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Transport of sulfonylurea herbicides from a barley field in Norway: Field and laboratory studies

Including development of an analytical method for sulfonylurea herbicides with LC-MS/MS

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Sammendrag

Til tross for at lavdosemidler har blitt brukt som ugressmidler i korndyrking verden over i snart 30 år, har det blitt utført få feltstudier av skjebnen til disse stoffene. Lavdosemidlene benyttes i så små doser og brytes så raskt ned at man har antatt at stoffene ikke utgjør noen risiko for vannmiljøet. Denne rapporten presenterer den første kontrollerte feltstudien av avrenning av lavdosemidler fra felt i Norge. Avrenning av lavdosemidler i lysimetersøyler i laboratoriet er også undersøkt. Rapporten gir i tillegg en beskrivelse av en metode for ekstraksjon og analyse av lavdosemidler i vannprøver med LC-MS/MS.

Summary:

Although the sulfonylurea herbicides have been used for many years worldwide, few field studies have been performed and little is known about the occurrence, fate and transport of sulfonylureas in the field. This report presents results from the first controlled field and laboratory-studies on the fate of sulfonylurea herbicides in Norway and a method for sample preparation and LC-MS/MS analysis of sulfonylurea herbicides in water samples is also presented.

Approved

Section Manager Børge Holen

Project leader

Marit Almvik

Preface

Although the sulfonylurea herbicides have been used for many years worldwide, few field studies have been performed and little is known about the occurrence, fate and transport of sulfonylureas in the field. This report presents results from the first controlled field- and laboratory-studies on the fate of sulfonylurea herbicides in Norway and a method for sample preparation and LC-MS/MS analysis of sulfonylurea herbicides in water samples is also presented. This one-year project was funded by the Norwegian Food Safety Authority as a part of the "Handlingsplan for redusert risiko ved bruk av plantevernmiddel (2004-2008)".

Due to the low doses applied, it has been assumed that the sulfonylurea herbicides have low potential for environmental effects on non-target organisms. Lack of sufficiently sensitive analytical methods has been a major obstacle in gaining information on the leaching of the herbicides. However, in the recent years, the development of LC-MS/MS technology has made possible the detection of pesticides at the nanogram-level in environmental samples.

The objectives of the present work were:

Main objective:

Evaluate the risk of transport of sulfonylurea herbicides in a barley field

Sub-objectives:

1. Develop an analytical method on LC-MS/MS for selected sulfonylureas and develop sample preparation methods for water and soil samples.

2. Assessment on the loss of sulfonylurea herbicides through surface and drainage water from a barley field.

3. Assessment of the leaching of sulfonylurea herbicides in soil columns.

The project has been a cooperation between Bioforsk Plant Health and Plant Protection (Marit Almvik, Randi Bolli, Agnethe Christiansen and Sven Roar Odenmarck) and the Norwegian University of Life Sciences, Department of Plant and Environmental Sciences (Gunnhild Riise and Trond Børresen). Kjell Wærnhus at Bioforsk performed the herbicide spraying of the Syverud field in 2007. In addition, representatives from the Norwegian Food Safety Authority (Cathrine Waage Tveit and/or Roger Holten) have participated in all project meetings.

Contents

1. Sammendrag

Metodeutvikling

Analyser av lavdosemidler i norske vannprøver har tidligere blitt utført ved Miljø Kemi i Danmark fordi analysemetode har manglet i Norge. Miljø Kemi har operert med kvantifiseringsgrenser på 0,01 µg/L. I dette prosjektet ble en analysemetode for tribenuronmetyl, amidosulfuron, metsulfuronmetyl, jodsulfuronmetyl og metabolittene INL5296 og INA4098 etablert på LC-MS/MS ved Bioforsk Plantehelse. Kvantifiseringsgrensen for morstoffene var 0,25 ng/L med metoden. Prøveopparbeidelsen av vannprøver inkluderte oppkonsentrering på fast fase kolonner med Strata X sorbenter med en gjenvinning på 60-120% for morstoffene og 35-45% for metabolittene. Høyere gjenvinning kan oppnås for metabolittene ved å bruke sorbenten Lichrolute EN. Stabiliteten av tribenuronmetyl og amidosulfuron ble undersøkt i vannprøver og i løsemiddel (acetonitril) for å kontrollere om herbicidene var stabile under lagring i fryser. Analysene viste at herbicidene var stabile i vannprøvene i hele stabilitetsstudiets varighet (4 måneder), og at de var stabile i acetonitril i minst 6 måneder.

Lysimeterstudier

Tribenuronmetyl, amidosulfuron, jodsulfuronmetyl og metabolitten metsulfuronmetyl ble påvist i utlekkingsvann fra 45 cm lange jordsøyler. Gjenfinningen av lavdosemidlene var lav i forhold til de appliserte konsentrasjonene. Gjenfinningen av tribenuronmetyl var 0,005 % og 0,009% for både amidosulfuron og jodsulfuronmetyl. Totalkonsentrasjonene av midlene påvist i vannprøvene var: 0,0016 µg/L tribenuronmetyl, 0,018 µg/L amidosulfuron, 0,0033 µg/L jodsulfuronmetyl (0,0047 µg/L inkludert metsulfuronmetyl). Beregning av GUS-indeksverdier for lavdosemidlene viste at mobiliteten følger følgende trend: INL5296 > Metsulfuronmetyl > Amidosulfuron > Tribenuronmetyl > INA4098 > jodsulfuronmetyl. Lysimeterstudiet viste at jodsulfuronmetyl kan være mer mobilt i Syverudjorda enn hva GUS-indeksen angir.

Resultatene for tribenuronmetyl kan imidlertid være feilaktige, da dette pesticidet også ble påvist i vann fra jordsøyler hvor stoffet ikke ble applisert. Årsaken til dette kan være at Syverudfeltet hvor jorda ble hentet fra, ble sprøytet med tribenuronmetyl året før og at rester av stoffet var tilstede i jorda ett år etter sprøyting. Utlekkingsstudiet med tribenuronmetyl bør repeteres og jorda som ble hentet fra feltet analyseres for eventuelle tribenuronmetyl-rester.

Den observerte utlekkingen av lavdosemidler i 45 cm lange jordsøyler indikerer at utlekkingsrisikoen kan være enda høyere i felt, hvor makroporer kan gi en mye raskere utlekking av vann og lavdosemidler enn det man kan måle i pakkede jordsøyler. Resultatene fra lysimeterforsøket må imidlertid sees i sammenheng med feltstudier.

Avrenning av lavdosemidler i felt

I denne delen av prosjektet, ble overflate- og drensavrenning av lavdosemidlene amidosulfuron og tribenuronmetyl undersøkt i kontrollerte ruteforsøk på Syverud, Ås i sørøst Norge. I tilegg til morstoffet ble det målt nedbrytningsprodukter av tribenuronmetyl, hhv. INL5296 og INA4098. Metsulfuronmetyl ble også påvist i vannprøvene. Metsulfuronmetyl er trolig et biprodukt i handelspreparatet (Express) eller et nedbrytingsprodukt av tribenuronmetyl.

Forsøksfeltet på Syverud har jord med stabil aggregatstruktur, stor infiltrasjonskapasitet og liten erosjonsrisiko. Rutene som inngikk i forsøket blir høstpløyd og vårharvet, og det ble foretatt vannproporsjonal prøvetaking av overflate- og drensavrenning. Forsøksperioden, juni 2007 - februar 2008, som helhet var svært nedbørsrik. Spesielt, juni og juli var nedbørsrike, hvor det kom 271 mm mot normalt 149 mm. For januar 2008 var det nedbørsrekord med 176 mm mot normalt 49 mm. Januar var også mild, noe som bidro til høy avrenning.

Både amidosulfuron og tribenuronmetyl ble målt i overflate og drensavrenning i konsentrasjoner som var godt over kvantifiseringsgrensen. Høyeste verdier var 0,31 µg/l og 0,08 µg/l for hhv. amidosulfuron og tribenuronmetyl. Disse maksverdiene ble registrert i en avrenningsepisode 20 dager etter sprøyting. Ved påfølgende avrenningsepisode, 8 dager senere, var konsentrasjonen av begge midlene vesentlig lavere. I overflateavrenningen, var det bare en avrenningsperiode om sommeren som ga tilstrekkelig mengde vann til måling av herbicider, 29. juli, nesten to måneder etter sprøyting. Konsentrasjoner av amidosulfuron og tribenuronmetyl var vesentlig lavere enn for maksverdiene i drensavrenningen med verdier på hhv. 0,2 µg/l og 0,001 µg/l.

Nedbrytningsprodukter av tribenuronmetyl ble også registrert i både overflate- og drensavrenning. I drensavrenningen var konsentrasjonen av morstoffet langt høyere enn for nedbrytningsproduktet i den første perioden rett etter sprøyting. Gradvis økte forholdet mellom nedbytningsproduktene og morstoffet tribenuronmetyl med tiden. I desember og januar var konsentrasjonen av INL5296 og INA4098 4-10 ganger høyere enn for tribenuronmetyl. I overflateavrenningen observerte vi et annet mønster. Konsentrasjonen av nedbrytningsproduktene var alltid høyere enn morstoffet tribenuronmetyl. Gjennomsnittskonsentrasjonen av tribenuronmetyl var også lavere for overflateavrenningen sammenlignet med drensavrenningen. Dette indikerer at nedbrytningen går raskere i øvre del av jordprofilet, både pga raskere mikrobiell omsetning og lavere pH som bidrar til mer effektiv hydrolyse av lavdosemidlene.

Det var et vesentlig større tap av lavdosemidler via drensavrenning sammenlignet med overflateavrenning pga. stor vanntransport gjennom drensgrøftene. Omlag 0,1 % av amidosulfuron og 0,2 % av tribenuronmetyl ble gjenfunnet totalt i avrenningen (sum av overflate + drensavrenning). Hvis nedbrytningsproduktene av tribenuronmetyl inkluderes, øker tapet for tribenuronmetyl til 0,4 %. Tapet av nedbrytningsprodukter var høyest vinterstid, noe som indikerer at et mildt vinterklima kan medføre økt avrenning av nedbrytningsprodukter. Nivåene av lavdosemidlene var lave, men verdiene ligger over drikkevannsgrensen på 0,1 µg/l for amidosulfuron (0,3µg/l) og i nærheten for tribenuronmetyl (0,08 µg/l).

Et av de viktigste funnene ved dette feltstudiet er lavdosemidlenes relativt store evne til å infiltrere en jordtype med stabil aggregatstruktur og relativt høyt innhold av organisk materiale ned til dypere deler av jordprofilet hvor nedbrytningen går saktere. Infiltrasjon ned til dypere jordlag hvor nedbrytningen går saktere, har stor betydning for lavdosemidlenes persistens i miljøet.

2. Abstract

Analytical method development

Sulfonylurea herbicides in Norwegian water samples have previously been analysed at Miljø-Kemi in Denmark, due to the lack of analytical method in Norway. Miljø-Kemi has a limit of quantification of 0.01 µg/L for these herbicides. In this project, we have established an analytical method for tribenuron-methyl, amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl and the metabolites INL5296 and INA4098 on LC-MS/MS at Bioforsk Plant Health and Plant Protection with a limit of quantification of 0.25 ng/L for the herbicides. These herbicides were selected based on their extensive use in cereals in Norway. INL5296 is a metabolite of tribenuron-methyl, whereas INA4098 is a metabolite of both tribenuron-methyl, iodosulfuron-methyl and metsulfuron-methyl. A sample preparation method for water samples was developed using Strata X solid phase sorbents for pre-concentration of the samples with a recovery of 60-120% for the herbicides and 35-45% for the metabolites. Higher recoveries (nearly 95%) of the metabolites can be gained by using Lichrolute EN sorbents. The stability of tribenuron-methyl and amidosulfuron were examined in water samples and in solvents (acetonitrile) in order to verify whether the herbicides are stable during storage in the freezer. The analyses showed that the herbicides were stable in the water samples during the entire study period (4 months), and stable in acetonitrile for 6 months.

Leaching of sulfonylurea herbicides in column studies

Tribenuron-methyl, amidosulfuron, iodosulfuron-methyl and the metabolite metsulfuron-methyl were found in drainage water from 45 cm soil columns after application of the herbicides. The recovery of the herbicides was low as compared to the initial amount applied (the total amount recovered was 0.005 % of added tribenuron-methyl, 0.009 % of added amidosulfuron and 0.009 % of added iodosulfuron-methyl). The actual total concentrations of herbicides in the drainage water was 0.0016 µg/L tribenuron-methyl, 0.018 µg/L amidosulfuron, 0.0033 µg/L iodosulfuron-methyl (0.0047 µg/L including metsulfuron-methyl). GUS index calculations classify the leachability of the compounds in the following order: INL5296 > Metsulfuron-methyl > Amidosulfuron > Tribenuron-methyl > INA4098 > Iodosulfuron-methyl. Our results show that iodosulfuron-methyl may be more prone to leaching in the Syverud soil than predicted by the GUS index.

However, the results for tribenuron-methyl may be questioned, as tribenuron-methyl and the metabolite INL5296 were detected in water fractions from columns were tribenuron-methyl had not been added. The reason for this is probably that the soil may have residues of tribenuron-methyl residues from spraying the previous year (2006).

The observed leaching below 45 cm of all three (four) sulfonylurea herbicides during the column study, may indicate that the risk of leaching may be even higher in the field, as field soil macropores can mitigate a more rapid flow of water and mobile pesticides than in repacked columns.

Sulfonylurea herbicides in surface runoff and drainage water– field experiments

Transport of amidosulfuron and tribenuron-methyl through surface and drainage water were studied in controlled field plots at Syverud, Ås, SE-Norway. Degradation products of tribenuron-methyl, i.e. metsulfuron-methyl, INL5296 and INA4098, were also monitored during the study. The Syverud soil has a relatively high content of nutrients, a high aggregate stability and infiltration capacity, but low erodibility. The field was sprayed with 58 g amidosulfuron/ha and 9 g tribenuron-methyl/ha on the 7th of June 2007. Water-proportional sampling of surface and drainage water was performed during the study period (June 2007 - February 2008). The two first months after spraying were very wet, with a total precipitation of 271 mm (normal precipitation mean is 149 mm).

The sulfonylurea herbicides amidosulfuron and tribenuron-methyl were measured in both surface runoff and drainage water at concentration well above the limit of quantification. Peak concentrations of 0.31 µg/l amidosulfuron and 0.008µg/l tribenuron-methyl were measured at the first significant flow event after application of the pesticides (20 days after spraying).

Due to low water volumes, surface runoff water was only measured once during the summer; in July, nearly two months after spraying. The concentrations of amidosulfuron and tribenuron-methyl in the surface runoff were 0.2 µg/l and 0.001 µg/l, respectively.

Transport of sulfonylurea herbicides through the drainage water was far more important than surface runoff, as the majority of water left the fields through the drains. The site received high amounts of precipitation and the sulfonylureas showed high mobility in the soils. Approx. 0.1 % of the amidosulfuron and 0.2 % the tribenuron-methyl (or 0.4% if the metabolites are included) applied was recovered in the water (total of surface runoff and drainage water).

In the drainage water, peak concentrations of both herbicides and degradation products were measured at the first significant flow episode, 20 days after application, whereas in the surface runoff water, the concentrations of the degradation products were seen to increase during the entire period, with maximum amounts in January. The degradation apparently proceeds faster in the upper soil layer, both as a result of higher microbial degradation and more efficient hydrolyses caused by lower pH in the upper compared to deeper layers of the soil profile. A climate with warmer winters may results in higher runoff of degradation products compared to winters where the soil is frozen.

The measured herbicide concentrations were low, but exceeded the drinking water limit (0.1 µg/l) for amidosulfuron (0.3 µg/l) and approached the limit for tribenuron-methyl (0.08 µg/l) 20 days after spraying.

An important knowledge from this study is that the sulfonylurea herbicides have a relatively high ability to infiltrate soil to deeper soil layers where the degradation proceeds slower and the mother compound can persist for a longer period of time.

3. Introduction

Sulfonylurea herbicides (i.e. sulfonylureas) are low dose herbicides (10-15 g a.i. ha^{-1}) used for pre- and post-emergence control of broadleaved and grassy weeds in cereals. Sulfonylureas were developed in the mid-1970s by DuPont and the fist sulfonylurea herbicide was introduced in Norway in 1984, and the sulfonylureas have subsequently more or less replaced the phenoxy herbicides (e.g. MCPA, mekoprop-p, dikchlorprop-p) and other herbicides used in cereals (fluroxypyr, bentazone and dicamba). The use of sulfonylurea herbicides in Norway was particularly stimulated through the introduction of tax rates on pesticide formulations, that are set according to the pesticides environmental and toxicological properties, and the price of sulfonylureas became very low compared to phenoxy herbicides and other herbicides (Netland and Wærnhus 2007). In the period 2003-2005, sulfonylureas were applied on 74% of the cereal areas in Norway. In 2008, six different sulfonylureas are used on cereals in Norway: tribenuron-methyl, metsulfuron-methyl, iodosulfuron-methyl, amidosulfuron, thifensulfuron methyl and mesosulfuron-methyl (Table 1 and 2). In addition, the sulfonylurea herbicide rimsulfuron is used in potato cropping.

Both the roots and foliage of plants can absorb sulfonylureas easily and plants can translocate sulfonylureas in both the xylem and phloem (Beyer *et al.*, 1988). The sulfonylureas work by inhibiting the plant-specific enzyme acetolactate synthase (ALS), which is required for the biosynthesis of branched-chain amino acids and eventually inhibit cell division at the root and shoot tips. Furthermore, sulfonylureas are ALS inhibitors solely and have no influence on other biochemical processes or a second site of activity. This site specificity can lead to the development of sulfonylurea-resistant weeds, which has been reported in *Stellaria media* (vassarve/Common chickweed), *Spergula arvensis* (linbendel/Corn spurry), *Galeopsis tetrahit* (kvassdå/ Common hemp-nettle), *Sonchus asper* (stivdylle/Spiny sow thistle) and Tripleurospermum inodorum (balderbrå/Scentless Mayweed) in Norway ((Netland and Wærnhus 2007).

The sulfonylureas are characterized by high biological activity on susceptible weeds, short half-lives and low toxicities to animal species. A high biological activity means that the amount of active ingredient applied to the environment is reduced and handling and container disposal issues are improved. The sulfonylureas show low toxicity to animals and humans, but are toxic to algae and extremely toxic to waterdwelling plants. According to the EU list of endpoint, the EC_{50} for the water plant *Lemna gibba* is: 9 µg/l amidosulfuron, 4 µg/l tribenuron-methyl, 10.000 µg/l INL5296 and 11.000 µg/l INA4098.

Low doses do not necessarily mean reduced risks, as the sulfonylureas are highly potent. Due to the sulfonylureas moderate to high mobility (Table 3) and increasing use, they may represent potential environment risks, especially for aquatic plants and microorganisms. Residual phytotoxicity to rotation crops may also be a problem. In Norway, tribenuron-methyl has been monitored in three streams (Mørdrebekken, Skuterudbekken and Kolstadbekken) in the period 1997-2002, but no residues have been found. Metsulfuron-methyl and thifensulfuron-methyl were monitored in the same streams in 2000-2001 with no findings. All analysis have been performed at Miljø Kemi in Denmark with quantification limits in the range of 0.01 – 0.03 µg/L (Ludvigsen og Lode 2008). The relative high limits of quantification and/or degradation of the sulfonylureas during transport may be parts of the reasons for the lack of findings. Tribenuron-methyl has been detected repeatedly in water samples in Sweden (Kreuger 1998). Neither tribenuron-methyl nor metsulfuron-methyl have been seen to leach to ground water in the Danish monitoring programme, but amidosulfuron has been detected several times in drainage water two meters below the soil surface, with a maximum concentration of 0.11 µg/L (Kjær *et al.* 2007).

Table 1. Approved sulfonylureas in Norway in 2008 (www.mattilsynet.no).

Approved sulfonylureas	Formulation	a.i. (g/kg)	Area (%) of cereals	Turnover in 2007 (kg a.i.)
Tribenuron-methyl	Express Harmony Plus 50T	512 176	31	885
Iodosulfuron-methyl	Hussar OD Hussar Chekker	100 g/L 50 12.5	16	372
Amidosulfuron	Gratil 75 WG Chekker Eagle 75 WG	750 125 750	2	262
Thifensulfuron-methyl	Harmony Plus 50T Harmony WSB	347 781	18	187
Metsulfuron-methyl	Ally Class 50 WG Ally 50 ST	100 521	5	195
Mesosulfuron-methyl	Atlantis WG	30	1	15
Rimsulfuron	Titus WSB	250	-*	95

Table 2. Turnover of sulfonylureas (kg active ingredient) in Norway 1996-2007 (www.mattilsynet.no).

Herbicid	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Tribenuron-methyl	744	806	2972	1124	5	504	758	717	721	783	876	885
Iodosulfuron-methyl	-	-	-	-	-	-	212	343	261	292	276	372
Amidosulfuron	-	65	281	432		117	153	270	365	198	222	262
Thifensulfuron-methyl	-	8	368	234		11	128	157	174	179	199	187
Metsulfuron-methyl	42	66	225	83		27	122	20	74	96	124	195
Mesosulfuron-methyl	-	-	-	-	-	-	-	-	-	28	16	15
Rimsulfuron	58	73	194	57		38	69	95	58	77	84	95
Pirimisulfuron-methyl	-	-	-	23	19	23	51	89	25	9	17	-
Chlorsulfuron	77	11	-	-	-	-	-	-	-	-	-	

3.1 Degradation of sulfonylureas

The fate of sulfonylurea herbicides in soils is directly related to their chemical structure and mainly to the ionisation of the sulfonylurea bridge (SO_2NHCON). Sulfonylureas are weak acids with pKa from 3 to 5, and in soils they exist mainly in the ionised (anionic) form. This explains their low sorption coefficients, which are pH-dependent. The degradation half-lives of the sulfonylureas tested can be regarded as short to moderate (Table 3).

Table 3. Sulfonylurea half-lives (days) in soil, according to the e-Pesticide Manual (2006).

Sulfonylurea	Half-life (days)
Tribenuron-methyl	2-12
Iodosulfuron-methyl	1-4
Amidosulfuron	3-29
Metsulfuron-methyl	11-51
Mesosulfuron-methyl	6-91
Thifensulfuron-methyl	2-6

Sulfonylurea herbicides degrade in soil primarily by microbial metabolism and aqueous hydrolysis, the latter is especially effective at low pH. The sulfonylurea bridge cleaves early in the degradation process, resulting in a triazine/pyrimidine amine (e.g. INL5296) and a sulphonamide (e.g. saccharin) (Figure 1). Increasing soil temperature and moisture tends to decrease the half-life of the herbicides, probably due to increased microbial degradation. It has also been reported that herbicide half-life is longer in soils with high organic matter content compared to soils with low content, presumably due to greater herbicide adsorption. Sulfonylurea herbicides are more likely to be persistent in alkaline soils (Barrett 1996).

In surface water, the sulfonylurea herbicides may undergo photodegradation in addition to microbial degradation and aqueous hydrolysis.

Sulfonylurea herbicides are also seen to degrade in alcohol (e.g. ethanol, methanol). Alcoholysis will not be of importance in the environment, but may be very significant during sample preparation and analysis.

Proposed degradation pathways of tribenuron-methyl and iodosulfuron-methyl are given in Figures 1, 2 and 3.

The degradation products are numerous, and only the most significant metabolites are shown.

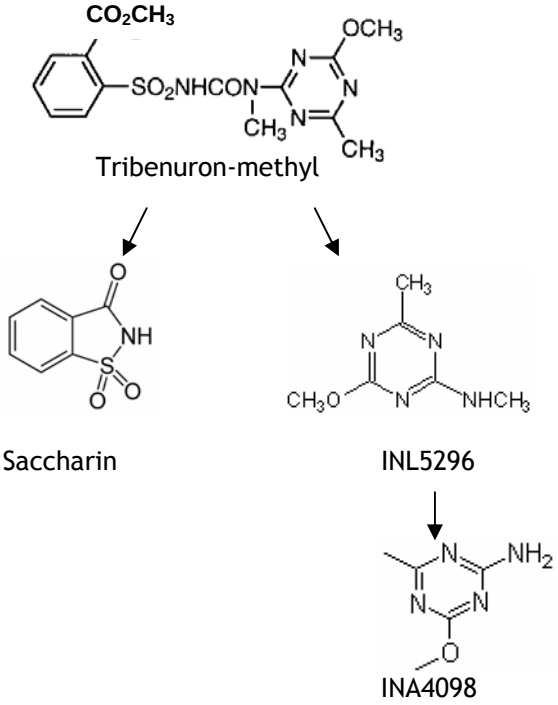

Figure 1. Proposed degradation pathway of tribenuron-methyl.

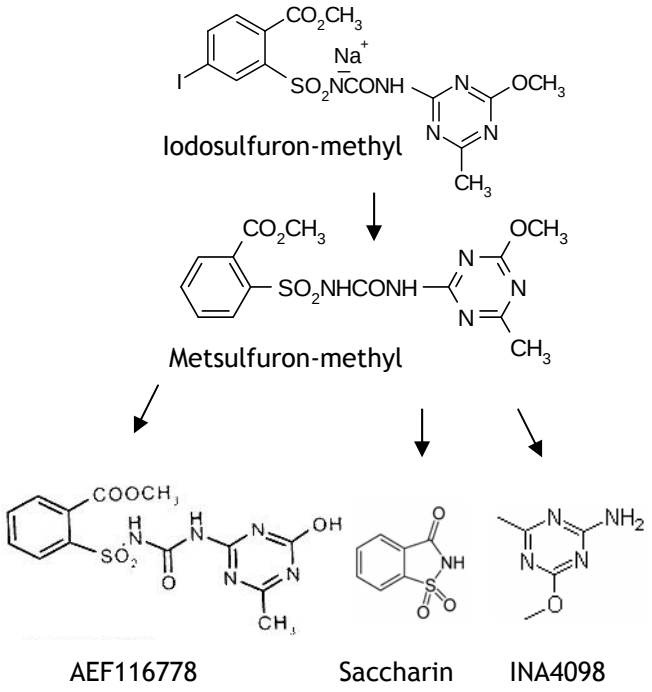

Figure 2. Proposed degradation pathway of iodosulfuron-methyl.

Although the degradation of several of the sulfonylurea herbicides is rapid, the complete mineralisation into CO_2 may require much longer time, as many metabolites are produced and some metabolites might be mineralised more slowly than they are formed (Andersen *et al.* 2001). This is also evident from the moderate to long half-lives of the degradation products INL5296, INA4098 and saccharin in Table 4. According to the classification of K_{oc}-values by Branham *et al.* (1995), INL5296 and INA4098 are highly to moderately mobile in soils, whereas AEF101630 is highly mobile in soils.

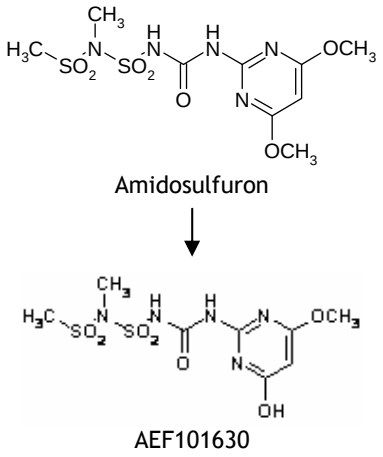

Figure 3. Amidosulfuron and the metabolite AEF101630

Table 4. Selected sulfonylurea metabolite half-lives (days) and K_{oc} (ml/g) in soil (Waage Tveit, 2007).

Degr. product	Parent herbicide	Half-life (days)	K_{oc} (ml/g)
INL5296	Tribenuron-methyl	148	53-138
INA4098	Tribenuron-methyl, Metsulfuron-methyl Iodosulfuron-methyl	22-240	46 - 226
Saccharin	Tribenuron-methyl, Metsulfuron-methyl Iodosulfuron-methyl	33-220	5.7 –10.6
AEF101630	Amidosulfuron	3-15	24 – 63

3.2 Leaching potential of sulfonylureas and metabolites

The mobility of the sulfonylureas in soil is largely influenced by organic matter content and soil pH, because the herbicides are weak acids that are negatively charged at neutral and alkaline pH. The acidic pK_a of the sulfonylureas is attributed to the acidic proton on the sulfonamide nitrogen (Beyer *et al.*, 1988). Acidic herbicides can bind organic matter by hydrophobic bonds, by hydrogen bridges, or by inorganic cation interactions (Laganá *et al.* 2000). Due to predominance of the anionic species at typical soil pH values and net negatively charged surfaces in soils, sulfonylureas typically have relatively low adsorption coefficients (K_{oc}) which increase with decreasing pH values and the herbicides have high to moderate mobility in soils (Table 5). K_{oc} measures the affinity for pesticides to sorb to organic carbon. The higher the value, the stronger the tendency to attach to and move with soil. A K_{oc} value of less than 100 indicates that a pesticide is very mobile in soils. A K_{oc} value between 100 and 1000 indicates that a pesticide is moderately mobile, and that mobility would be determined by other factors such as soil type and persistence. A K_{oc} value of 1000 or more usually indicates that a pesticide is immobile (Branham *et al.*, 1995).

Table 5. Adsorption coefficients and water solubility (at pH 7 unless otherwise stated) for sulfonylurea herbicides (K_{oc}; sorption per unit organic C, ml/g) (e-Pesticide Manual (2006) and Waage Tveit (2007)).

Sulfonylurea	Solubility in water (mg/l)	K_{oc} (ml/g)
Tribenuron-methyl	2040	10 - 74
Iodosulfuron-methyl	25000	43
Amidosulfuron	9.0 (pH6) 13000 (pH10)	2.6 - 82
Metsulfuron-methyl	2800	4 – 60
Mesosulfuron-methyl	483	26 – 345

The vast majority of sulfonylurea herbicide metabolites have a high potential to leach to ground water. Both triazine (e.g. INL5296 and INA4098) and pyrimidine amines ((e.g. some degradates of amidosulfuron, mesosulfuron and rimsulfuron) tend to be the most persistent of all sulfonylurea metabolites (Barrett 1996). Triazine and pyrimidine amines vary widely in their sorptivity. Pyrimidine amines especially, have a strong affinity for organic matter and clays, with K_{oc} values ranging up to several hundred ml/g.

4. Analytical method development

Marit Almvik, Bioforsk

4.1 Development of extraction method for water samples

Introduction

Extraction of multiple pesticides from an environmental sample can be a difficult task if the analytes exhibit different physico-chemical properties (polarity, acidity, solubility and stability etc.). The problem is often encountered during development of extraction methods for pesticides and their metabolites, as the metabolites normally are more polar or hydrophilic than their mother compounds. It may then be a challenge to find an extraction solvent or to find a solid phase extraction sorbent that will work for all the analytes. For the extraction of sulfonylurea herbicides and metabolites from water samples, solid phase extraction (SPE) has become the method of choice, as SPE combines both extraction and enrichment of the analytes in the water samples (Figure 4). As compared with conventional liquid-liquid extraction SPE exhibits lower costs, reduced processing time and solvent consumption.

An array of SPE sorbents have been applied for the enrichment of sulfonylurea herbicides from water: C_{18} bonded silica, polystyrene-divinylbenzene (PSDVB) (e.g. Lichrolut EN, Isolute ENV+), surface-modified PSDVB (e.g. Strata X, Strata X-CW), divinylbenzene-N-vinylpyrrolidone copolymers (e.g. Oasis HLB). Perreau *et al.* (2007) found that metsulfuron-methyl, thifensulfuron-methyl and INA4098 were better retained on Lichrolute EN (>85% recovery) than on Oasis HLB or Bond-Elut C18. On the other hand, Polati *et al.* (2006) found that the recovery of amidosulfuron, thifensulfuron-methyl and tribenuron-methyl were too low (<74%) on Lichrolut EN, but found good recoveries (>81 %) with Strata RP-18 or Strata X sorbents. The varying results using Lichrolute EN can be attributed to the properties of the pesticides and pH-control during sample preparation. Metsulfuron-methyl (pK_a = 3.75) is best retained on an EN-column at acidic conditions (Perreau *et al.* 2007), whereas tribenuron-methyl (pK_a = 5.0) is rapidly hydrolysed at acidic conditions and SPE-enrichment need to be performed at neutral pH, as done by Polati *et al.* (2006).

Figure 4. SPE-cartridge with a Strata-X sorbent.

Extraction and enrichment with SPE: As the water sample is loaded onto and through the cartridge, the pesticides in the water are retained by the sorbent. Afterwards, a small volume of solvent is applied to detach or elute the pesticides from the sorbent.

A sample preparation method for the selected sulfonylureas that are approved in Norway (tribenuron-methyl, amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl and the metabolites INL5296, INA4098 and AEF101630) was not available. The purpose of this part of the study was to develop a sample preparation method with SPE that could be applicable for all the 7 analytes.

Materials and methods

Chemicals and reagents

Tribenuron-methyl (99%), amidosulfuron (97.5%), metsulfuron-methyl (99%) and iodosulfuron-methyl (89%) were obtained from Dr. Ehrenstorfer GmbH (Germany). INL5296 (2-Methoxy-4methyl-6-(methylamino)-1,3,5,-triazine) (96%) and INA4098 (2-amino-4-methoxy-6-methyl-1,3,5-triazin) (97%) were obtained from Aldrich (France), whereas AEF101630 (3-(4-hydroxy-6-methoxypyrimidin-2-yl)-1-(N-methyl-N-methylsulfonyl-aminosulfonyl)-urea) was obtained from Bayer (Germany). Internal standard metsulfuron-methyl D3 (triazine methoxy deuterium-3 labelled) was obtained from Dr. Ehrenstorfer GmbH (Germany). Acetonitrile and methanol (both from SDS) were Pestipure grade, ultrapure water was prepared from a Millipore MilliQ system (USA). All standards were dissolved in acetonitrile. Solutions of amidosulfuron and AEF101630 were prepared by sonication-activated dissolution. Calibration standards for LC-analysis were prepared in methanol directly prior to analysis.

The sorbents tested in the solid phase extraction of water samples were Lichrolute EN (Merck, Germany), Isolute ENV+ and Isolute C18 (IST, Germany), Oasis HLB (Waters, USA), Strata X, Strata X-CW and Strata X-AW (Phenomenex, USA) and SupelMIP triazine 10 (Sigma-Aldrich, USA/MIP Technologies, Sweden).

Solid phase extraction

Milli-Q water fortified at a level of 0.2 µg/ml was used for the recovery studies with HPLC-UV analysis, whereas Milli-Q water fortified with 0.005 or 0.2 ng/ml was used for the recovery studies using LC-MS/MS. For acidified water samples (100-200 ml, pH 3 or 5 with acetic acid), the sorbents were conditioned with 3 ml methanol and 3 ml acidified water (1.5% v/v acetic acid). For neutral water samples the sorbents were conditioned with 3 ml methanol and 3 ml water. The water samples were percolated through the cartridges at drip-flow. The sorbents were eluted with 2 or 4 ml acetonitrile or acidified acetonitrile (2% v/v formic acid). The SupelMIP triazine 10 sorbent was conditioned with 1 ml methanol, 1 ml water and 1 ml $NH_4H_2PO_4$ (25 mM, pH 3) and eluted with 3x1 ml acetonitrile. The eluates were evaporated to dryness under a gentle stream of nitrogen and redissolved in 0.5 ml methanol and analysed immediately.

HPLC-UV analysis

An Agilent 1100 HPLC-DAD system with a model G1354A quaternary pump, a G1313A autoinjector, a G1315A diode array detector, a G1322A vacuum degasser was used. The injection volume was 20 µl. Separation was conducted on a Waters SunFire C18 column (100 x 4.6 mm i.d., 3.5 µm) at room temperature. The mobile phase was methanol and acidified water (0.05% v/v acetic acid) at a flow of 1 ml/min and a gradient of 5-80% methanol in 15 minutes. Quantitation was performed at 230 nm using external standard calibration.

LC-MS/MS analysis

A Water Alliance 2695 LC-system with binary pump, degasser and autosampler with cooling of samples down to 4°C was used. The LC was equipped with an Alltech Alltima C18 HPLC column (2.1 mm x 150 mm, 5.0 µm particle size) for sample separation. The ionisation and detection system consist of Quattro Ultima Pt triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionisation source. The mobile phase was methanol and 5 mM formic acid in water at a flow of 0,3 ml/min and a linear gradient of 10-95% methanol in 6 minutes, then hold 95 % methanol for 10 minutes, before returning to initial conditions. Total runtime was 18 min. The parameters for ionisation and detection are listed in appendix 3.

Results and discussion

Initial studies with 4 sulfonylurea herbicides and various sorbents

An initial recovery study of the four herbicides (tribenuron-methyl amidosulfuron, metsulfuron-methyl and iodosulfuron-methyl) was performed with the following sorbents: Oasis HLB (200 mg), Isolute C18(EC) (200 mg), Lichrolut EN (200 mg) and Isolute C18/ENV+ (400 mg). The Milli-Q water samples were either pH-adjusted (pH 3) or non-adjusted (pH 6). An acidic pH of 3 was tested because this pH level was below the pK_a of all the studied sulfonylurea herbicides (iodosulfuron-methyl has the most acidic pK_a; 3.22). Figure 5 shows the recoveries from this experiment.

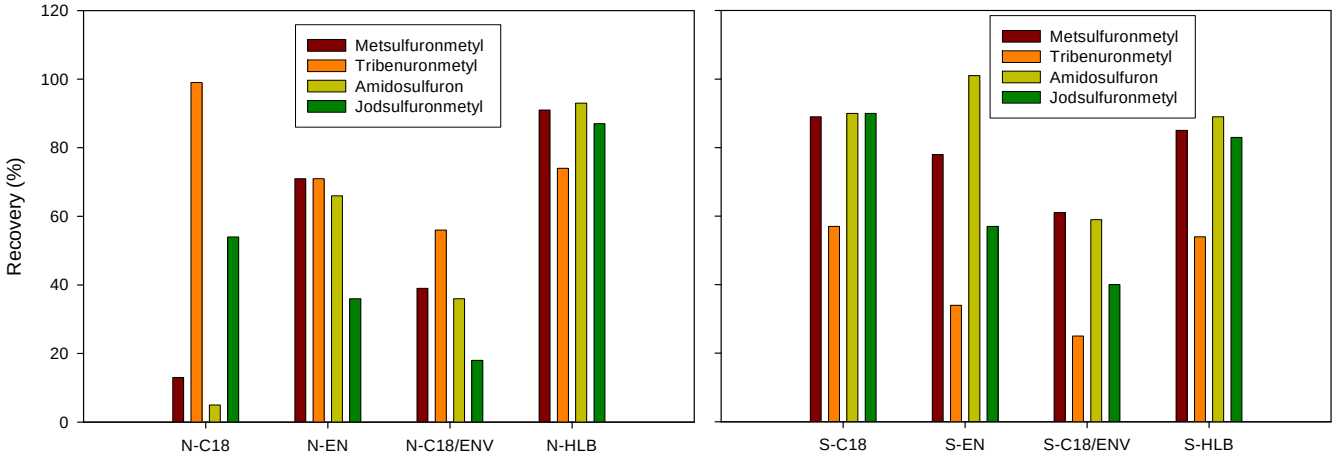

Figure 5. Recovery (%) of sulfonylurea herbicides using C18, EN, C18/ENV and HLB sorbents with neutral (N) water samples (left) or acidified water, pH 3 (S) (right).

The figure shows that the best recoveries are gained with the HLB (neutral water) and C18 (acidic water) supports. Metsulfuron-methyl (pK_a = 3.75) and amidosulfuron (pK_a = 3.58) are poorly retained on the C18 support at pH 6, but highly retained at pH 3 - a fact which can be attributed to the compounds being in a protonated state at pH < pKa and therefore more readily retained by non-polar mechanisms on the C18 sorbent. On the opposite, tribenuron-methyl (pK_a = 5) is much better recovered at pH 6 than at pH 3 on all sorbents. Bossi *et al.* (1998) observed the same effect; poor recovery of tribenuron-methyl on C18-support at pH 4.5, and concluded that tribenuron-methyl rapidly undergo hydrolysis even in slightly acidic solutions. Therefore, acidification of water samples should be avoided.

Initial studies with 4 sulfonylurea herbicides and 3 metabolites

The metabolites INL5296, INA4098 and AEF101630 were included together with the 4 herbicides in this recovery study, using Lichrolut EN (200 mg), Isolute ENV+ (200 mg) and Oasis HLB (200 mg) sorbents. The Lichrolut EN was tested because it was assumed that the EN sorbent would retain the metabolites well, as observed for INA4098 by Perreau *et al.* (2007). The Isolute ENV+ sorbent was tested because it is a hydroxylated polymer and thus expected to retain the more polar metabolites.

As the first study showed that 3 of the herbicides were better retained at acidic pH in the water sample, a slight pH-adjustment to pH 5 was also tested; anticipating a compromise between the recovery of tribenuron-methyl and the other herbicides.

The Isolute C18 support was also tested, but gave poor recovery of the metabolites (data not shown).

Figure 6 shows that the highest recoveries are gained with the Oasis HLB sorbent, with recoveries > 83% for all analytes, except INA4098, which has a recovery of only 40%. Oasis HLB is a hydrophilic polymeric sorbent made from a balanced ratio of two monomers; the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone and should therefore be able to retain a wide range of analytes. The

hydrophilic-lipophilic balance of the Oasis HLB sorbent probably allows a stronger retention of the ionised sulfonylurea herbicides (at pH 6) than the other sorbents. Another important parameter is the low pore size (8 nm) of the Oasis HLB sorbents. Oasis HLB appears to be the most extensively used sorbent for the extraction of polar compounds and especially acidic compounds (Reemtsma and Quintana 2006). The polar functional groups on the sorbent is assumed to increase the wettability of the sorbent. But during this study, a serious problem with the Oasis HLB cartridges was the percolation of water. To percolate pure Milli Q-water at a volume of 100 ml was very time-consuming. This raises concerns about the feasibility of percolating even higher volumes of real field samples.

The metabolite AEF101630 was not retained on any of the sorbents. The Lichrolut EN sorbent retained the metabolites INA4098 and INL5296 well (recovery >88%), but the recovery of tribenuron-methyl decreased rapidly from pH 6 to pH 5 (from 62% till 50% recovery, respectively), whereas amidosulfuron and iodosulfuron are poorly retained at pH 6. It was difficult to find optimum conditions for the extraction of the sulfonylurea herbicides with the EN sorbent. The EN sorbent works well for the retention of metsulfuron-methyl and INA4098, as also described by Perreau *et al.* (2007), but not for tribenuron-methyl and iodosulfuron-methyl. It appears that the pesticide sorption on the EN sorbent is dominated by non-polar retention mechanisms that is favoured at a pH were tribenuron-methyl is rapidly hydrolysed.

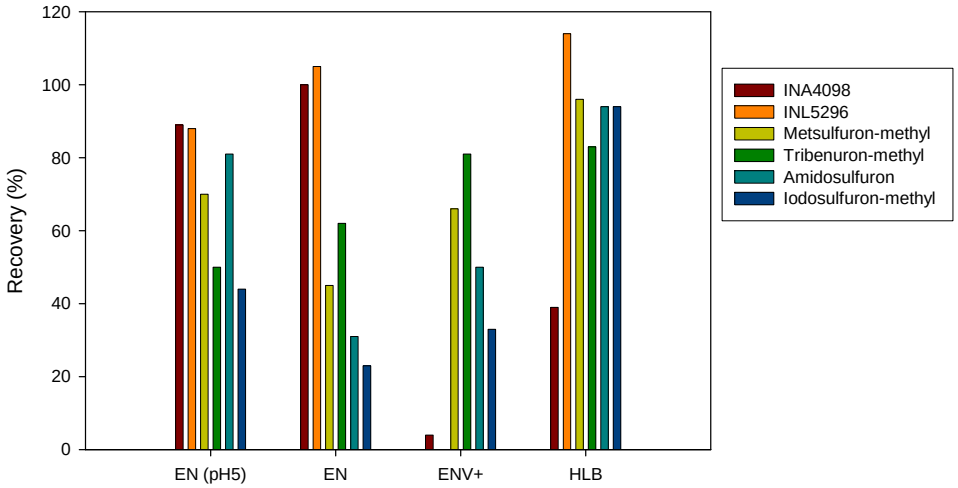

Figure 6. Recovery (%) of sulfonylurea herbicides and metabolites using EN, ENV+ and HLB sorbents. Water with pH 5 was used for one EN support; otherwise non-adjusted water was used (pH 6). The metabolite AEF101630 was not recovered from any of the sorbents and is not shown.

The metabolites were not well retained on the Isolute ENV+ sorbent. The better adsorption on Lichrolut EN compared to Isolute ENV+ can be explained by the lower particle and pore size and the higher surface area of the EN sorbent (Table 6).

Table 6. Parameters (sorbent surface area, sorbent pore size and sorbent particle size) for the studied polymeric SPE sorbents. See Appendix 2 for more details.
PVP-DVB = poly(N-vinylpyrrolidone-divinylbenzene), PS-DVB = polystyrene.divinylbenzene.

Sorbent	Polymer type	Surface area (m^2/g)	Pore size (nm)	Particle size (µm)
Lichrolut EN	PS-DVB	1200	3	40-120
Oasis HLB	PVP-DVB	830	8	30-100
Isolute ENV+	Hydroxylated PS-DVB	1000	85	10-140
Strata X	Pyrrolidone PS-DVB	800	8.5	33
Strata X-CW	Carboxylic pyrrolid. PS-DVB	800	8.5	33
SupelMIP	Molecular imprinted for triazines			

The metabolite AEF101630 is produced when amidosulfuron is O-demethylated, rendering a metabolite with a hydroxylic group that makes it very hydrophilic (Figure 3). Such highly polar compounds are very difficult to extract from water with present SPE sorbents. None of the tested SPE sorbents in the initial studies were able to retain AEF101630. Therefore, a new and emerging class of SPE sorbents was tested: the MIPs.

A molecular imprinted polymer (MIP) is a polymer that is formed in the presence of a molecule that is extracted afterwards, thus leaving complementary cavities behind. These polymers show a certain chemical affinity for the original molecule. The functional mechanism is similar to antibodies or enzymes. These polymers should thus have a high selectivity and produce very clean extracts.

Study with SupelMIP triazine 10 sorbents

The "SupelMIP triazine 10" is designed for analytes containing a triazine (a ring containing three nitrogens). All the sulfonylurea herbicides and metabolites tested in this study contain a triazine ring, except amidosulfuron and AEF101630, which have a diazine ring (only two nitrogens). Nevertheless, the recovery study shows that amidosulfuron was well retained (80% recovery) on the SupelMIP triazine 10 sorbent (Figure 7). INL5296 and INA4098 were recovered at low rates (11% and 24%, respectively), and AEF101630 not at all. Obviously, AEF101630 is too hydrophilic to be retained, even on this very selective sorbent. The SupelMIP triazine 10 sorbents are very expensive and cannot be used regularly for routine purposes. Another obstacle is that the sorbent is only intended for low samples volumes (< 10 ml) with decreased recoveries at higher volumes.

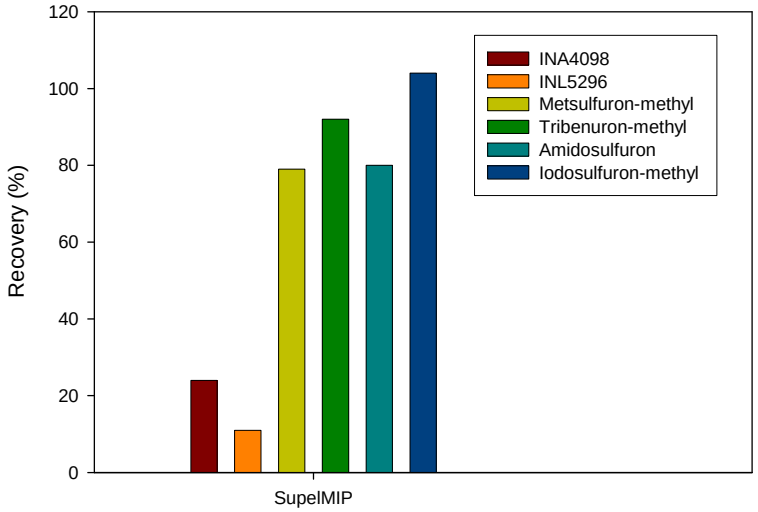

Figure 7. Recovery (%) of sulfonylurea herbicides and metabolites using SupelMIP triazine 10 sorbent with a sample volume of 10 ml. The AEF101630 metabolite was not recovered and is not shown.

A Chromabond EASY sorbent (a modified sorbent with a weak anion-exchange group) was also tested for the retention of the herbicides and INL5296 and INA4098. The sorbent did not retain any of the herbicides, but gave recoveries of 65% for the two metabolites. AEF101630 was not included in the test, but the fact that only the metabolites were retained, may imply that this sorbent work better for hydrophilic compounds.

Study with Strata sorbents

Polati *et al.* (2006) found that the Strata X sorbent gave good results for tribenuron-methyl and amidosulfuron, so a study was performed with the sulfonylurea herbicides and metabolites (2). The Strata X sorbent is a styrenic skeleton modified with a pyrrolidone group. According to the manufacturer, Strata X offers numerous retention mechanisms including hydrophobic, hydrogen bonding and π-π, providing "near universal" selectivity for acidic, neutral and basic drugs (www.strataspe.com).

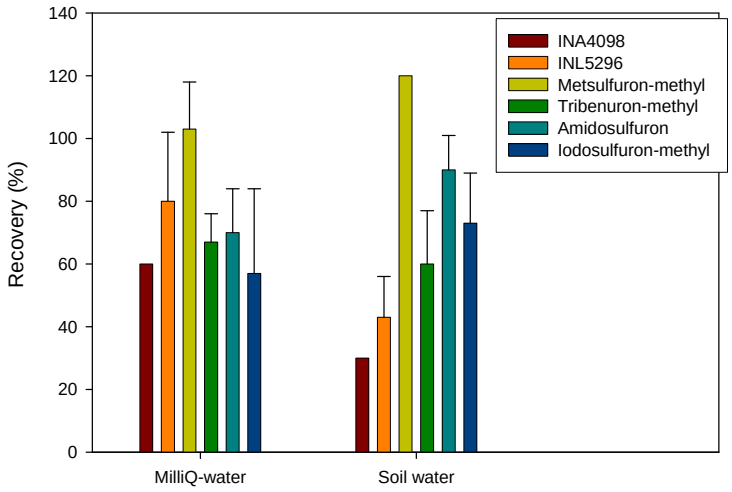

Figure 8. Recovery (%) of sulfonylurea herbicides and metabolites using Strata X sorbent. The water samples were fortified Milli Q-water or soil leachate water from the Syverud field.

The herbicides and the two metabolites were well recovered from the ultrapure water (58-102%), but with lower recoveries for the metabolites in the soil water samples (Figure 8). The latter could be due to sorption to soil particles in the water.

The mixed-mode Strata sorbents Strata X-AW (weak anion-exchange with a diamine group) and Strata X-CW (weak cation exchange with a carboxylic group) were also tested, and gave similar results as with Strata X (data not shown). Strata X was preferred over these ion-exchange sorbents, because Strata X can be eluted with pure solvent, whereas the others must be eluted with basic or acidic solvents that may initiate hydrolysis of the herbicides.

Conclusions

The best results overall were obtained with the Oasis HLB and the Strata X sorbents. The metabolites INL5296 and INA4098 were however best recovered on the Lichrolut EN support. None of the sorbents retained the very hydrophilic metabolite AEF101630.

The Strata X sorbent was preferred over Oasis HLB because the Strata X sorbents could be used with higher sample volumes than the Oasis HLB and was more time-efficient. Also, the costs of Strata X were half of the costs of Oasis HLB.

A possible solution for getting higher recoveries of the two metabolites may be to stack the Lichrolut EN cartridge on top of the Strata X cartridge, but this may lead to longer percolation times during sample loading to the cartridges.

4.2 Development of extraction method for soil samples

There was not time to develop a sample preparation method for soil samples during the project period, but the issue is discussed and a method suggested here:

Different solvents have been employed to extract sulfonylurea herbicides from soil: 0.1M $NaHCO_3$ (pH 8.5) (Galletti *et al.* 1995), 0.1 M KH_2PO_4/methanol pH 8 (50:50) (Laganá *et al.* 2000), 0.01 phosphate-buffered saline (PBS) pH 7.4 (Andersen *et al.* 2001) and PBS-acetonitrile (80:20) (Bossi *et al.* 1999). Bernal *et al.* (1997) found that extraction of tribenuron-methyl from soil with pure organic solvents was poor, with only 53% recovery using acetonitrile. Therefore, aqueous buffer-solvent solutions should be applied. Laganá *et al*. (2000) argues that aqueous methanol should be used instead of aqueous acetonitrile due to the inefficiency of acetonitrile to compete for desorption as acetonitrile has a low capacity for forming hydrogen bonds. Aqueous methanol is able to attach itself quite strongly via hydrogen bonds to the acidic adsorption sites of the organic matter. This gives better desorption and higher recoveries for metsulfuron-methyl and thifensulfuron-methyl than aqueous acetonitrile. Sabadie (2000) observed that the alcoholysis of metsulfuron-methyl appeared totally inhibited in a buffered aqueous ethanol solution at pH 7. Thus, a buffered hydro-alcoholic solution may be the extraction solvent of choice for sulfonylureas in soil.

Considering that the sulfonylurea herbicides are weak acids with dissociation constants (pKa) ranging from 3-5, and that the stability of some depends strongly on pH, the use of solvent systems with pH>pKa should increase the solubility of the compounds in soils. At this pH value the dissociated anions of the herbicides should be poorly distributed in organic soil because of its higher water solubility and its possible repulsion due to the surface negative charge of the organic matter (Laganá *et al.* 2000). However, a very basic aqueous extraction will liberate many acidic soil interferences e.g. humic and fulvic acids. Therefore, a solution pH slightly above 7 would be preferred.

Extraction temperatures for metsulfuron-methyl and thifensulfuron-methyl should not exceed 60°C (Font *et al.* 1998).

4.3 Development of HPLC-DAD method

Marit Almvik, Bioforsk

Introduction

A HPLC-DAD method for analysis of tribenuron-methyl, amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl, INL5296 and INA4098 was developed in order to analyse extracts from the initial sample preparation studies. Subsequently, a LC-MS/MS was installed at the laboratory, and an analytical method was developed on the LC-MS/MS (see chapter 4.4), as this instrument offers much lower detection limits than the HPLC-DAD. Minimum concentrations for sulfonylurea herbicide standard solutions for HPLC-UV-DAD detection are about 1 ppm or 1 µg/ml, whereas LC-MS/MS can detect the herbicides at a concentration of 0.1 ng/ml, i.e. 10.000-fold lower than LC-DAD.

Materials and methods

Chemicals and reagents

Same as the "Materials and methods" section in chapter 3.1.

Instrumentation

An Agilent 1100 HPLC-system with a model G1354A quaternary pump, a G1313A autoinjector, a G1315A diode array detector and a G1322A vacuum degasser was used. Separation of the analytes was first tested on an Agilent Eclipse XDB-C18 column (150 x 4.6 mm, 5 µm) and then on a Waters SunFire C18

(100 x 4.6, 3.5 µm) column. The injection volume was 20 µl and the analytes were detected at 230 nm with a bandwidth of 16 nm. The final mobile phase was methanol in water with 0.05% acetic acid (pH 3.4) and a gradient from 5 to 80 % methanol in 15 minutes at a flow of 1 ml/min.

Results and discussion

Selection of detection wavelength

Pure standard solutions of each herbicide/metabolite were injected and the UV-spectra taken by the DA-detector in order to determine the wavelength were the maximum absorbance occurred for each compound. Tribenuron-methyl has UV_{max} at 233 nm, amidosulfuron at 238 nm, metsulfuron-methyl at 225 nm and iodosulfuron-methyl at 225 nm. INL5296 has UV_{max} at 225 nm and INA4098 at 216 nm. The final detection wavelength was set to 230 nm for all the analytes.

Selection of analytical separatory column

An Agilent Eclipse XDB-C18 was fist tested, but the column did not separate tribenuron-methyl and iodosulfuron-methyl when 45% acetonitril in acidic water was used as a mobile phase, so a SunFire C18 column with lower particle size was tested. The SunFire column was able to separate all the herbicides.

Selection of mobile phase

The sulfonylurea herbicides are weak acids and exist in their protonated (neutral) state when pH in the solvent is low (pH<3.2). The water in the mobile phase was kept acidic (pH 3.4) by the addition of 0.05% acetic acid. Both acetonitrile and methanol were tested, but methanol was finally chosen as co-solvent, due to improved peak shape of the metabolites.

The following gradient elution programme was set up for the separation of the metabolites and herbicides (B = water with 0.05% acetic acid):

0-2 min: 5% methanol in B

2-6 min: linear gradient from 5 to 54% methanol in B

6-8.5 min: hold 54% methanol in B

8.5-15 min: linear gradient from 54-80% methanol in B

3 minutes post-run in order to equilibrate to initial mobile phase conditions

Injection solvent effect on peak shape

The peaks of the metabolites INL5296 and INA4098 were split when the standard/sample was dissolved in acetonitrile. This occurred both when acetonitrile or methanol was used as co-solvent in the mobile phase, due to the strong solvent effect. Injections could not be performed with mobile phase as a solvent, because the low pH would cause hydrolysis of the analytes. A weaker solvent had to be used. The peak shape of INL5296 was optimal when the standard was solved in methanol. The peak height of INA4098 was higher when dissolved in methanol, but the peak was fronted. The peak shape of INA4098 was improved when solved in a basic solution; i.e. 5% NH_4OH in methanol. These results may indicate that the metabolites are basic compounds. Basic compounds demonstrate better peak shapes at pHs above their pK_a values. The metabolites also have functional groups with pK_a values at 10-11 (CH_3NH: pK_a of 9.8, CH_2NH_2: pK_a of 10.6) which indicate that they are basic compounds.

4.4 Development of LC-MS/MS method

Sven Roar Odenmarck og Agnethe Christiansen, Bioforsk

Introduction

Sulfonylurea herbicides are used in much lower concentrations and degrade more rapidly compared to other older herbicides (Ayano *et al.*, 2004). Therefore, they occur at very low concentration in environmental samples. Most modern pesticides are characterized by medium to high polarity and thermal stability that makes liquid chromatography (LC) the favoured analytical separation technique. Coupling of LC to tandem mass spectrometry (MS/MS) with electrospray ionisation (ESI) i.e. LC-ESI-MS/MS, offers high sensitivity at the sub-µg/L level (Gervais *et al.*, 2008). Analytical method development is done for optimization of sensitivity and specificity, gaining lower limit for quantification and confirmation for the compounds analysed. The following compounds were included in the work:

Tribenuron-methyl, amidosulfuron, metsulfuron-methyl, iodosulfuron-methyl, INL5296, INA4098 and AEF101630.

The MS system works at high vacuum, hence compounds in the liquid state in the LC-system need to be introduced into the MS-system via an electrospray (ES) interface. In the ES the compounds are first ionized in the capillary, and then the solved ions are sprayed in a fine mist (small droplets) and dried by a stream of heated nitrogen gas (Figure 9). The vapour formed is pumped out to maintain high vacuum in the system and the sample cone extracts ions based on predetermined parameters (specific polarity and strength of the cone voltage (CV)). The MS/MS consist of two quadrupoles (named MS1 and MS2) separated by a collision cell. Only ions with predetermined specific mass to charge ratios (*m/z*) are passed through the first quadrupol. These "parent ions" are then collided with argon in the collision cell and fragmented into smaller ions ("daughter ions"). Ions with specific predetermined *m/z* ratios are allowed through the second quadrupole (MS2). The specific ES and MS/MS parameters are tested and optimized for each compound in order to give high sensitivity and specificity.

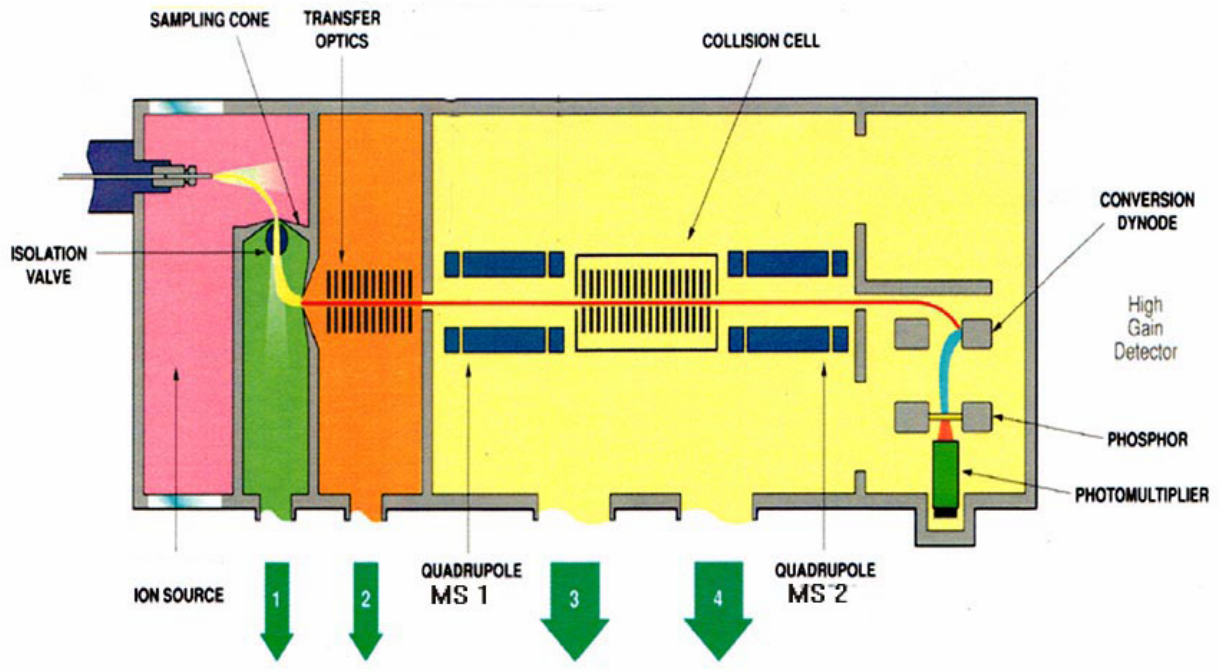

Figure 9. Schematic diagram of an ESI- MS/MS-detector.

Method development on LC-MS/MS is divided in two parts:

1. MS/MS-method for ionization and detection of sulfonylurea herbicides and metabolites.

2. LC-method for separation of sulfonylurea herbicides and metabolites.

The MS/MS-method is the first step in the process, as detection of the compounds is needed for evaluation of the results from the LC-separation development.

Materials and methods

Chemicals and reagents:

Ultra pure water was produced by a Milli-Q system (Milli-pore, USA). Acetonitrile (SDS), methanol (SDS) and formic acid (98-100%, Merck) were of analytical grade (Pestipur).

Instrumentation:

A Water Alliance 2695 LC-system with a binary pump, degasser and autosampler with cooling of samples down to 4°C was used. The LC was equipped with an Alltech Alltima C18 HPLC column (2.1 mm x 150 mm, 5.0 µm particle size) for sample separation. The ionisation and detection system consisted of Quattro Ultima Pt triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionisation source.

MS/MS-method for ionization and detection of sulfonylurea herbicides and metabolites

The optimization of ionization in the ES was done by running the "QuanOptimise" feature and the capillary set to 3.0 kV. The compounds were made in separate solution at 1.0 µg/ml in acetonitrile. The solutions were injected and run on LC with a mobile phase of methanol: water (80:20), isocratic elution. All compounds were tested for ionization with electrospray in positive (ES+) and negative (ES-) mode. The cone voltage (CV) was tested in the range 30 – 75 V (increase of 7 in 8 steps) and the collision energy (CE) was tested in the range 8 – 30 eV (increase of 4 in 7 steps).

LC-method for separation of sulfonylurea herbicides and metabolites

Various compositions of the mobile phase were tested:

Milli-Q water or Milli-Q water with 5 mM acetic acid mixed with either methanol, acetonitrile or methanol: acetonitrile (50:50)

The effect of the sample solvent on peak performance was tested and the following solvents were used: acetonitrile:Milli-Q water, methanol (MeOH), acetonitrile (AcN) and methanol with 5% ammonium hydroxide (NH_4OH).

Limit of quantification (LOQ): The LOQ were set to the level were the compound signals were well defined from the background noise, and where recovery showed good repeatability.

Results and discussion

MS/MS-method for ionization and detection of sulfonylurea herbicides and metabolites.

All compounds gave best response in the positive electrospray mode (ES+), except the metabolite AEF101630, which can be detected in the negative mode (ES-) only. It was determined to exclude AEF101630 from further method development, as the compound would need a separate analytical method and because the sample preparation development showed that the compound had to be extracted with a separate method, too.

The selected mass-transitions (parent – daughter-ion), optimized CV and CE parameter for each compound are listed in appendix 3.

LC-method for separation of sulfonylurea herbicides and metabolites.

The stability of sulfonylurea herbicides is shorter in methanol than in acetonitrile (due to alcoholysis), but methanol gives better chromatography. Methanol was therefore selected as sample solvent. However, an important pre-requisite was that the samples and calibration standards had to be analysed the same day as prepared. Calibration standards (6) were prepared in the concentration range of 0.0001-0.01 µg/ml in methanol.

The optimum mobile phase composition (best sensitivity and separation of the compounds) was obtained with methanol and 5 mM formic acid (HCOOH) in Milli-Q water.

Mobile phase: C: Methanol

B: 5 mM HCOOH

Gradient: 0-6 min: 10 % to 95 % methanol in B

6-16: 95% methanol in B

16.1-18: 10 % methanol in B

Sensitivity: The sensitivity of the metabolites was lower than for the sulfonylurea herbicides.

The limit of quantification for the compounds were:

Tribenuron-methyl, amidosulfuron, metsulfuron-methyl and iodosulfuron-methyl: 0.0001 µg/ml

INL5296 and INA4098: 0.0010 µg/ml.

Conclusion

The developed LC-MS/MS method offers good sensitivity and specificity for the analysis of the selected sulfonylurea herbicides and metabolites in this project. The sensitivity of the metabolites is poorer than for the mother compounds due to their nature as smaller molecules and more affected by interferences and noise. This method can easily be extended to include new sulfonylurea herbicides.

5. Stability study of water samples and standards

Marit Almvik, Bioforsk

Introduction

Sulfonylureas undergo hydrolysis in aqueous media at a rate dependent upon both temperature and pH (Table 7). The pH of the solution controls the rate of hydrolysis since the neutral form of the sulfonylurea bridge is considerably more susceptible to hydrolysis than the ionic form (Brown, 1990). In the presence of water the sulfonylurea bridge is cleaved, resulting in a triazine/pyrimidine amine and a sulfonylamide or a sulphonamide. The sulfonylurea herbicides studied in this work, are predominantly stable at pH 7-9 (10) and of these, the most unstable compounds are tribenuron-methyl and amidosulfuron. With a pKa of 3.58, amidosulfuron is >99% ionized at pH > 5.6, whereas tribenuron-methyl is >99% ionized at pH > 7.

Table 7. Sulfonylurea herbicides hydrolysis half-life (days) at 25°C (e-Pesticide Manual, 2006).

Sulfonylurea	Hydrolysis half-life (days) at 25°C
Tribenuron-methyl	Stable at 8-10, but rapid loss a pH<7.
INL5296	Stable (pH 5)
Iodosulfuron-methyl	365 (pH 7)
Amidosulfuron	34 (pH 5), 365 (pH 7)
Metsulfuron-methyl	22 (pH 5). Stable at pH 7-9.
Mesosulfuron-methyl	253 (pH 7)
Thifensulfuron-methyl	1.5 (pH 5), 10 (pH 7)

The water samples from the Syverud field (Chapter 6) were stored in the freezer at -20°C for several months prior to analysis. In order to verify if any hydrolysis of the sulfonylurea herbicides (tribenuron-methyl and amidosulfuron) occurred during storage, a stability study of these herbicides in ultrapure water and in drainage water was performed.

Materials and methods

Chemicals and reagents

Tribenuron-methyl, amidosulfuron and D3-labelled metsulfuron-methyl were obtained from Dr. Ehrenstorfer GmbH, Germany. Stock standards were prepared in acetonitrile, whereas a fortification standard mix of tribenuron-methyl and amidosulfuron was dissolved in MilliQ-water. Calibration standards for LC-analysis were prepared in MilliQ-water directly prior to analysis.

Fortification of water

MilliQ-water (pH 6.2) and drainage water (pH 7.6) from the Syverud field were fortified with tribenuron-methyl and amidosulfuron at a level of 0.01 µg/ml. Internal standard metsulfuron-methyl-D3 was added at a level of 0.01 µg/ml. The fortified water samples (50 ml) were transferred into plastic bottles, but also into several HPLC-vials of glass and capped. The vials were stored at -20°C. The samples were analysed on LC-MS/MS at various time intervals.

Analysis with LC-MS/MS

See "Materials and methods" section in Chapter 3.1.

Results and discussion

Stability of tribenuron-methyl and amidosulfuron in water during frozen storage

The MilliQ-water had lower pH (6.2) than the soil water (7.6), hence, degradation through hydrolysis was expected to be more pronounced in the MilliQ-water. The water samples were analysed during the period March to July, and the results showed that there were no degradation of amidosulfuron and tribenuron-methyl in the soil water during the 4 months period (Table 8). Some variability in the concentrations measured was found, possibly due to variations in the calibration standards used for each analysis. A slight decline (6%) in the tribenuron-methyl concentration is observed in MilliQ-water, but no degradation of amidosulfuron was observed. The levels of INL5296, which is a metabolite of tribenuron-methyl, does not clearly indicate any production of INL5296 in the MilliQ-water during the study. It is therefore not known if the slight decline in the tribenuron-methyl level is due to degradation or to day-to-day variability in the analysis.

Table 8. Concentration (µg/ml) of amidosulfuron, tribenuron-methyl and INL5296 stored in MilliQ-water and soil water in the freezer during March - July 2008. Fortification of the water samples at a level of 0.01 µg/ml was performed the 18th of March.

Water type	Date	Amidosulfuron	Tribenuron-methyl	INL5296
MilliQ-water	18. March 2008	0.0092	0.0099	0.0003
	2. April 2008	0.0104	0.0082	0.0005
	24. July 2008	0.0103	0.0092	0.0004
	30. July 2008	0.0107	0.0093	0.0007
Soil water	18. March 2008	0.0096	0.0101	0.0003
	2. April 2008	0.0098	0.0090	0.0002
	24. July 2008	0.0101	0.0100	0.0002
	30. July 2008	0.0106	0.0095	0.0004

These results show that the herbicides in the Syverud drainage water samples were probably not prone to hydrolysis during storage in the freezer.

However, it is recommended that the pH of water samples are measured before storage, and pH adjusted to > 7, if necessary.

Stability of stock standards

Stock standards of tribenuron-methyl and amidosulfuron were prepared in acetonitrile and stored in the freezer. New stock standards were regularly prepared. Analysis of these stock standards shows that these herbicides are stable in acetonitrile during frozen storage for at least 6 months.

Conclusions

The stability studies of tribenuron-methyl and amidosulfuron show that the compounds are stable when dissolved in both drainage water and acetonitrile and stored frozen (< -18°C) for at least 4 and 6 months, respectively.

6. Leaching of sulfonylureas in soil columns

Randi Bolli, Bioforsk

Introduction

Mobility, microbial metabolism and aqueous hydrolysis are the most important processes which determine the fate of sulfonylureas in the environment. The extent of sorption to soil influence the mobility and hence the bioavailability and leaching risk in a soil – water system. The sulfonylurea herbicides have a moderate to high mobility (The e-Pesticide Manual, 2006) and may therefore represent a possible environmental risk for contamination of groundwater and surface water.

The purpose of this study was to investigate the leaching potential of three sulfonylurea herbicides and one metabolite under controlled laboratory conditions. This study was conducted according to OECD guidelines 312 "Leaching in soil columns".

Materials and methods

Test substances

Tribenuron-methyl, amidosulfuron, iodosulfuron-methyl and the metabolite INA4098 were used in this study. Tribenuron-methyl was formulated as Express (500 g tribenuron-methyl/kg), amidosulfuron was formulated as Gratil 75 WG (750 g amidosulfuron/kg) and iodosulfuron-methyl was formulated as Hussar (50 g iodosulfuron-methyl/kg). IN-A4098 is a metabolite which appears from both tribenuron-methyl and iodosulfuron-methyl. In the soil column study pure standard was used for INA4098. For the quantitative analysis, pure standards (>89 %) of these pesticides were purchased from Dr. Ehrenstorfer GmbH, Germany.

The amount of pesticide applied corresponded to the normal area dose (NAD). Express has a NAD of 0.015 kg/ha, Gratil 75 WG has a NAD of 0.08 kg/ha and Hussar has a NAD of 0.20 kg/ha. This corresponds to 5.9 µg tribenuron-methyl/column, 47 µg amidosulfuron/column and 7.9 µg iodosulfuron-methyl/column, respectively.

The tracer, potassium bromide (KBr), was applied at the same time as the pesticides in order to follow the transport of water. An amount of 78.5 mg KBr was applied to each column corresponding to 52.7 mg Br/column. This is in accordance with the amount applied at the field study.

Soil sampling

The soil was sampled from three different layers at Syverud, based on previous soil profile characterisation (Appendix 4). The samples were taken from 0 – 10 cm, 10 – 25 cm and 25 – 45 cm. The soil was sieved through a 4 mm screen and stored at 4 °C until the study started. An overview of the grain size distribution and organic carbon content in soil from Syverud is given in Appendix 4.

Leaching study

Preliminary leaching study

A preliminary leaching study was conducted with tribenuron-methyl and KBr, using one soil column, in order to get an indication of the tribenuron-methyl front; i.e. in which fraction the pesticide was expected to come. This was meant to give an indication of when the sulfonylurea herbicides were expected to be found in the leachates. The preliminary study was executed in the same way as the full study.

Leaching study

Columns made of stainless steel with an inner diameter of 10 cm and heights of 50 cm were used in the test. 14 columns were packed in layers which corresponded to those sampled at the field. In order to achieve a uniform packing of the soil columns, the columns were weighed after packing. The soil columns were pre-wetted with artificial rain (0.01 M $CaCl_2$) in order to displace the air in the soil pores by water. Thereafter, the excess water was drained off by gravity before the test substances were applied to the soil columns. To obtain a homogeneous distribution of the solutions, the substance was applied evenly across the surface of the soil columns. Tribenuron-methyl was added to soil columns no. 1, 2 and 3, the metabolite IN-A4098 was added to columns no. 4, 5 and 6, amidosulfuron was added to columns no. 7, 8 and 9 and iodosulfuron-methyl was added to columns no. 10 and 11. Soil column no. 12 was a control column where no pesticides were added. The soil surfaces were then covered by a round filter paper to ensure an even distribution of the rain over the soil surface. Artificial rain was added drop-wise to the soil columns with the help of a peristaltic pump at a speed of approximately 1 ml/min which corresponded to approximately 280 mm rainfall during 48 hours. Eight water samples/fractions were collected; 10, 14.5, 19, 23.5, 34, 38.5, 43 and 47.5 hours after application of the pesticides. The expected volumes of these samples were 500 ml for the fractions after 10 and 34 hours, while for the six other fractions the expected volume was 200 ml. For each sample, approximately 25 – 30 ml was taken out for bromide analysis. pH was measured before the samples were stored in the freezer until sample preparation and analysis. The temperature in the lysimeter laboratory was set to 10°C during the study.

The lysimeter laboratory Photo: E. Fløistad

Extraction and analysis

Pesticides: The sulfonylurea herbicides were extracted as described in the chapter "Development of extraction method for water samples". The instrument method is described in the chapter "Development of LC-MS/MS method".

Bromide: Bromide concentrations from each fraction were determined in room-tempered solutions using a bromide specific ion electrode (Orion Research, Boston MA). Calibration of the electrode was performed with external bromide standards in the range of 0.43 – 128.2 mg/L.

GUS index

The GUS index (Groundwater Ubiquity Score Index) assesses the leachability of molecules and the possibility of finding these compounds in groundwater (Gustafson, 1989). It is calculated by the following equation:

GUS = log (soil DT_{50}) x (4 – log K_{OC})

This index is based on the parameters (1) mobility in soil, given by the organic carbon partition coefficient (K_{OC}), and (2) soil persistence, given by the degradation half-life in soil (DT_{50}). Pesticides with GUS > 2.8 are considered potential leachers (L), those with GUS between 1.8 and 2.8 have transient properties (T) and those with GUS < 1.8 are considered to be non-leachers (NL).

Results and discussion

Preliminary study

The preliminary study was conducted with tribenuron-methyl and KBr in order to estimate when the sulfonylurea herbicides were expected to be found in the leachates. Eight water fractions were collected and analyzed for tribenuron-methyl and bromide.

Bromide

The highest amount of bromide was measured in fraction five, approximately 34 hours after application (figure 10). The amount added to the soil column was 52.7 mg bromide and the total amount recovered was 77 %.

Tribenuron-methyl

Tribenuron-methyl was found in six of the eight collected water fractions. The highest amount of pesticide was found in fraction three, approximately 19 hours after application (figure 10). The amount added to the soil column was 5.9 µg tribenuron-methyl, but the total amount recovered was less then 0.2 % of the initial amount applied. Tribenuron-methyl has a degradation half-life in soil of 2 – 12 days and hydrolysis is rapid when pH is less than 7 (The e-Pesticide Manual, 2006). In this preliminary study pH was adjusted in each fraction with ammonium bicarbonate (NH_4HCO_3) to pH 7 – 8 before freezing. Within this range tribenuron-methyl is supposed to be stable. This became a problem during the sample preparation because the ammonium bicarbonate salt led to precipitation reactions in the water phase and caused clogging of the SPE-columns used for extraction. Therefore, pH adjustment of samples was omitted in the main experiment.

Low recovery (<0.2 %) from the soil column can be explained by a combination of very fast degradation in soil, a quick hydrolysis in the water fraction (pH of the water fraction was approximately 4.8) and sorption to soil. It was expected that bromide, which is supposed to follow the water, should be in

front of tribenuron-methyl, whereas the opposite was observed (Figure 10). This deviation between bromide and tribenuron-methyl may reflect differences in sorption affinity to soil surfaces.

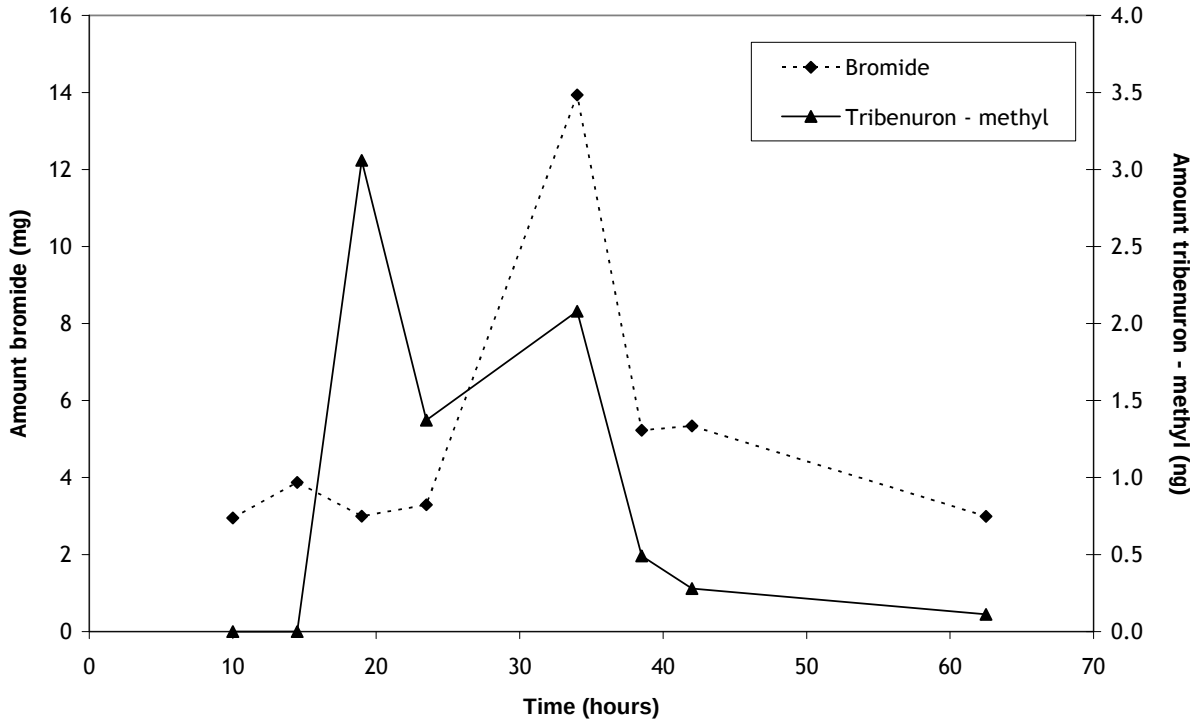

Figure 10. Leaching of bromide and tribenuron-methyl in the preliminary study

The column study

Based on the preliminary study it was expected that the highest amount of bromide and pesticides would come 19, 24, 34 and 39 hours (fraction 3 – 6) after application.

Bromide

Table 9 shows the total leaching of water and bromide in the different soil columns. The total amount of leaching water varies between approximately 1900 ml and 2500 ml. This is due to the peristaltic pumps not delivering exactly the same amount of water to each column. It can also be a result of variety in the packing of columns (i.e. water holding capacities), although the soil columns had nearly similar weights.

Table 9. Total leaching of water (leachate volume) and bromide in soil columns from the lysimeter study

Soil column no.	1	2	3	4	5	6	7	8	9	10	11	12
Amount of water (ml)	2320	2323	2082	1896	2448	2528	1906	2013	1986	2184	2036	2150
Amount of bromide (mg)	27.4	37.4	32.6	16.8	33.4	50.3	28.6	43.2	17.0	39.7	30.8	36.4
Recovery of added bromide (%)	51.9	70.8	61.8	31.8	63.3	95.3	54.2	81.9	32.2	75.2	58.4	68.9

The total amount of bromide in the leachates also differs between the columns. Bromide is a tracer of the water flow and table 9 shows that the columns with the lowest water volumes also have the lowest amounts of bromide in the leachates. It can be seen that a leachate volume of approximately 2500 ml

is necessary to give a nearly 100 % recovery of bromide. This suggests that the water fractions should have been collected according to volume and not according to time during the study.

Tribenuron-methyl

Tribenuron-methyl formulated as Express, was applied to soil column no. 1, 2 and 3 with an application rate at 5.9 µg active ingredient/column. Figure 11 shows the result from column 3, since there were no detections of tribenuron-methyl and metabolites in the water from column 1 and 2.

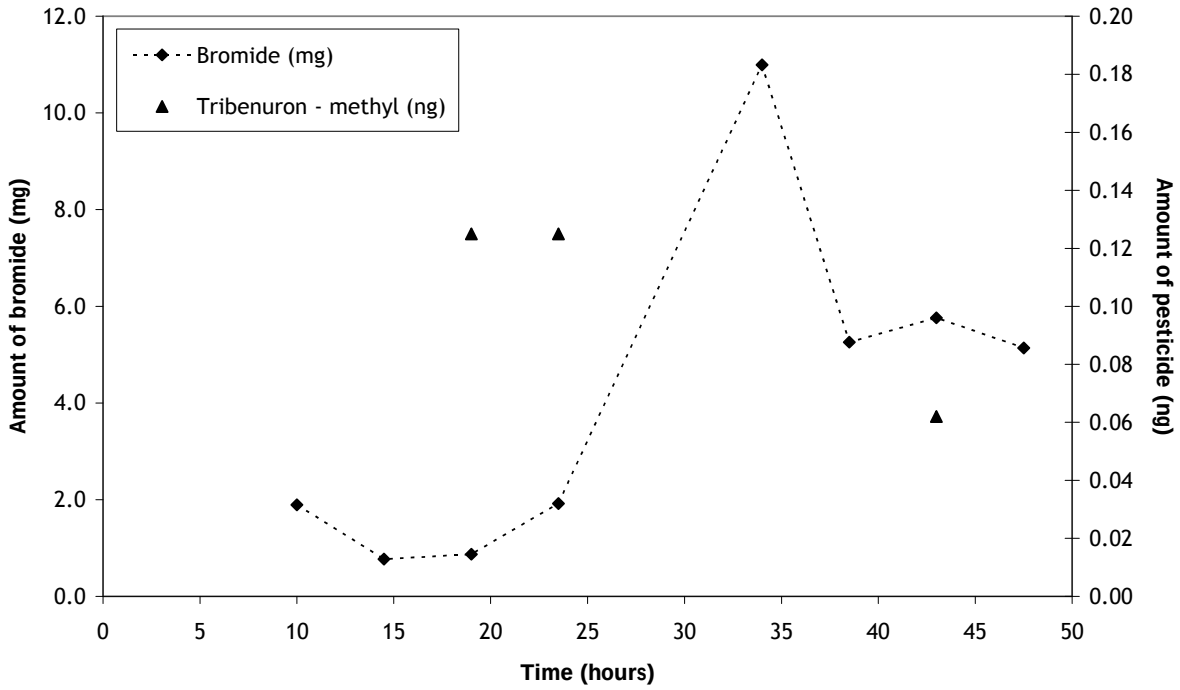

Figure 11. Leaching of bromide and tribenuron-methyl from soil column no. 3

Tribenuron-methyl was found in three of eight collected water fractions from column 3. The highest amount of pesticide was found in fraction three and four, approximately 19 and 24 hours after application (Figure 11). The total amount recovered was 0.005 % of added tribenuron-methyl. Tribenuron-methyl has a DT_{50} value in soil at 2 - 12 days, and it also hydrolyses very rapidly at a pH less than 7. Tribenuron-methyl primarily degrades by aqueous hydrolysis and by microbial degradation, where the main degradation products are INL5296 (90 %) and INA4098 (8 %). Soil pH affects the hydrolysis, being faster in acidic than in alkaline soils (The e-Pesticide Manual, 2006). The water fractions had a pH around 4 – 5. This can cause a very fast hydrolysis during collection of water before freezing. The stability test (Chapter 5) showed that hydrolysis during storage in the freezer is probably not an issue. As in the preliminary study, the highest bromide amounts were seen to leach out in fraction five, and after elution of tribenuron-methyl. This reflects, as mentioned earlier, differences in sorption affinity to soil surfaces.

Tribenuron-methyl and the metabolite INL5296 were also detected in three other columns (no. 6, 8 and 9) where tribenuron-methyl was not applied. INA4098 was added to column 6 and amidosulfuron was added to columns 8 and 9. In columns 6 and 9, tribenuron-methyl was detected in relatively small concentrations in two and three fractions, respectively. In column 8, tribenuron-methyl was found in three of eight fractions while its metabolite INL5296 was detected in all eight fractions (Figure 12). The many detections of both tribenuron-methyl and INL5296 in water from column 8 implies that the soil must have contained tribenuron-methyl and/or INL5296 at rather high concentrations, since INL5296 was not detected in any of the columns 1,2 and 3, where tribenuron-methyl was positively

added to the soil. The most reasonable explanation for the detections in columns 6, 8 and 9 is that the soil contained residues of tribenuron-methyl and INL5296 from application of tribenuron-methyl (Express) to the Syverud field in June 2006. When we sampled the soil for the soil column study in June 2007, we were not aware that the field had been sprayed with tribenuron-methyl the previous year, otherwise the soil would have been analysed for residues of the pesticide before initiation of the soil column study. Tribenuron-methyl has a very short half-life and it is surprising that the pesticide is still present in the field soil one year after spraying.

It is also surprising that tribenuron-methyl is detected in only 4 of the 12 soil columns, as the soil was mixed and sieved and should be homogenous and contain the same amount of tribenuron-methyl (if the residues origin from spraying of the field). The rapid flow of solutes in columns 6 and 8, as reflected by the high recovery of bromide (> 82%, see Table 9), may explain the detection of tribenuron-methyl in the leachate from these columns. The detection of tribenuron-methyl in only one of the three columns were it was added (no. 1, 2 and 3) also reflects that there were varieties in the packing and sorption capacities of the soil columns.

Detection of tribenuron-methyl and INL5296 in water samples from soil columns where tribenuron-methyl was not added, seriously influences the interpretation of the results for column no. 3. The soil from the field needs to be analysed for content of tribenuron-methyl and INL5296 in order to confirm any presence of pesticide residues from previous spraying at the field. The column leaching study ought to be repeated for tribenuron-methyl in order to conclude as to the leaching of tribenuron-methyl.

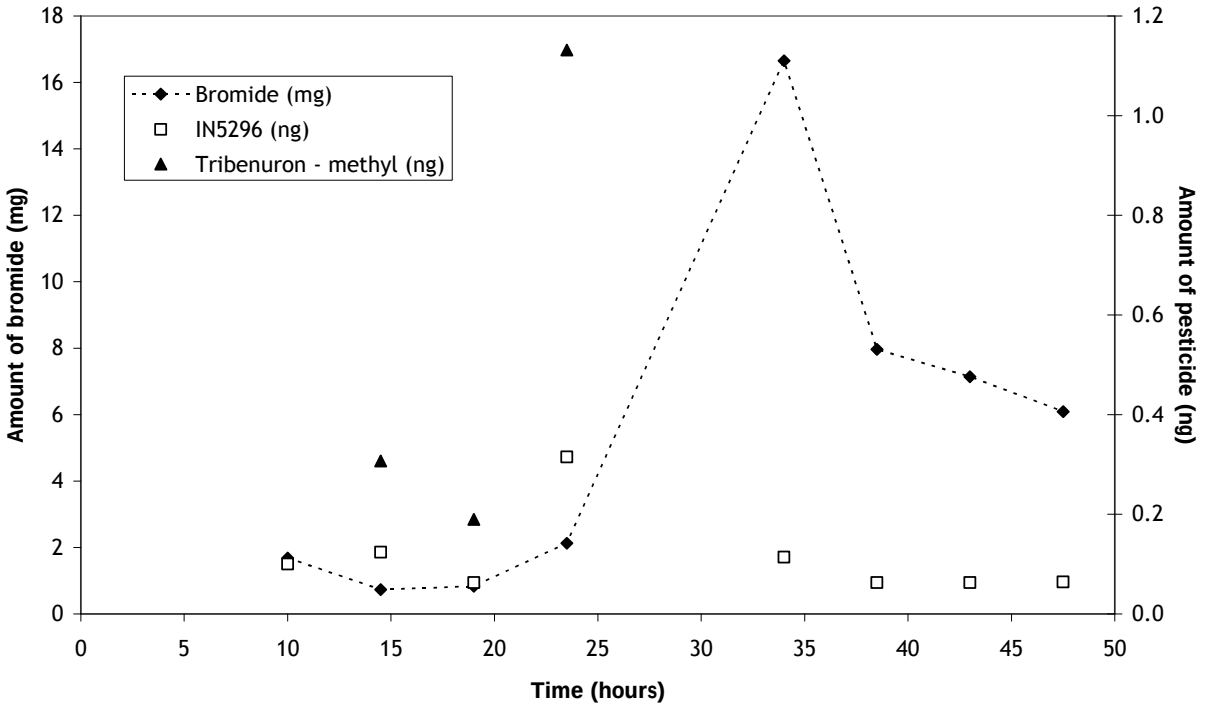

Figure 12. Leaching of bromide, tribenuron-methyl and INL5296 from soil column no. 8 (amidosulfuron application only).

INA4098

INA4098 is a degradation product of both tribenuron-methyl and iodosulfuron-methyl. For this soil column study pure standard was used and it was applied to soil column nr. 4, 5 and 6 with an application rate at 5.9 µg/column. Even though INA4098 is more persistent in soil than its mother compounds, having a half-life in soil of 22 – 240 days, there was no detection of INA4098 in the water from these columns. INA4098 is also stronger adsorbed to the soil than its mother compounds (see Table 4 and 5). The limit of detection for INA4098 was 10 times higher than for the mother compounds and the residual amounts of INA4098 were probably very low and not detectable.

Amidosulfuron

Amidosulfuron, formulated as Gratil 75 WG, was applied to soil column no. 7, 8 and 9 with an application rate at 47 µg active ingredient/column. Figure 13 shows the result from column 9. Amidosulfuron was not detected in drainage water from columns 7 and 8.

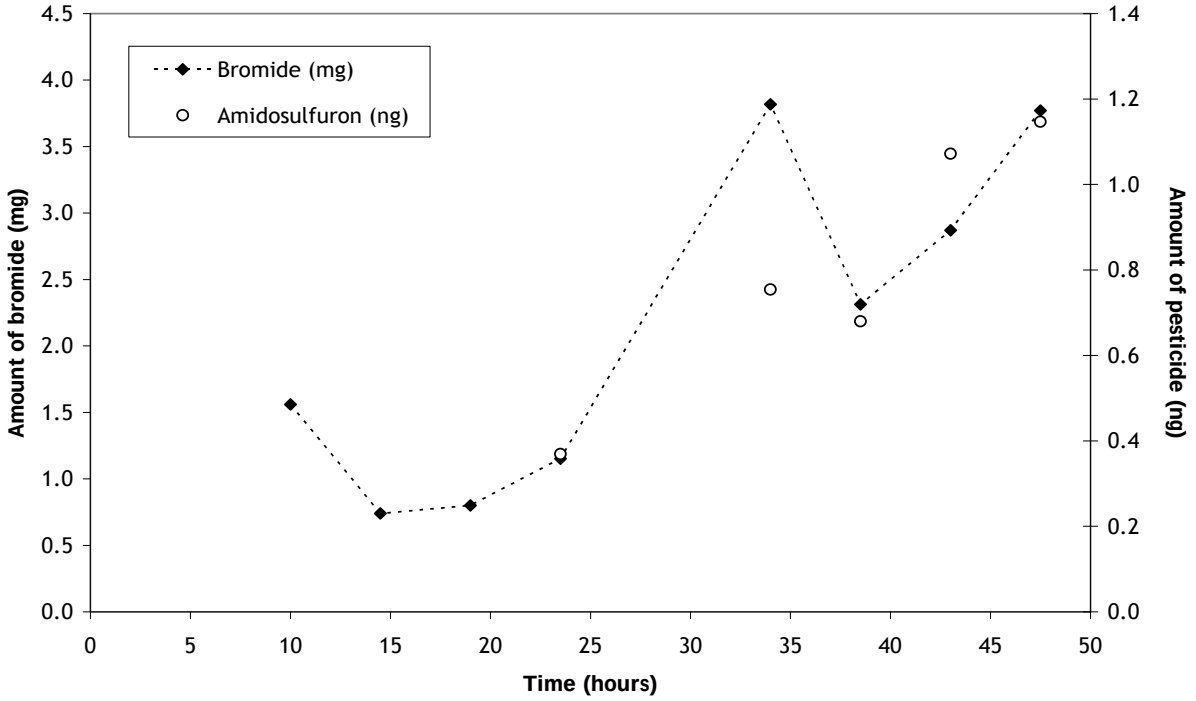

Figure 13. Leaching of bromide and amidosulfuron from soil column no. 9

Amidosulfuron has a longer half-life and is also less adsorbed to soil than tribenuron-methyl. Amidosulfuron was found in five of eight water fractions from column 9. The highest amount of pesticide was found in fraction seven and eight, approximately 43 and 48 hours after application (figure 4). The total amount recovered was 0.009 % of added amidosulfuron. According to figure 4, the amount of amidosulfuron is increasing at the end of study. The recovery might have been higher if more water samples had been taken out at the end of the experiment. Amidosulfuron has a DT_{50} value in soil of 3 – 29 days and a DT_{50} value in water of 34 days at pH 5. The pH value in the water fractions were approximately 4 – 5. Amidosulfuron mainly undergoes a microbiological degradation and according to the e-Pesticide Manual, the degradation is independent of the pH value, but dependent on the biological activity in soil. In this column, only 32 % of added bromide was recovered and it is therefore be strange that amidosulfuron was detected in this column. The explanation may be non-uniform packing of the soil column and that bromide and amidosulfuron have different flow patterns and different affinities to soil.

Iodosulfuron-methyl

Iodosulfuron-methyl, formulated as Hussar, was applied to soil column no. 10 and 11 with an application rate of 7.9 µg active ingredient/column. Figure 14 shows the result from column 11. There were no detection of iodosulfuron-methyl and metabolites in the water from column 10.

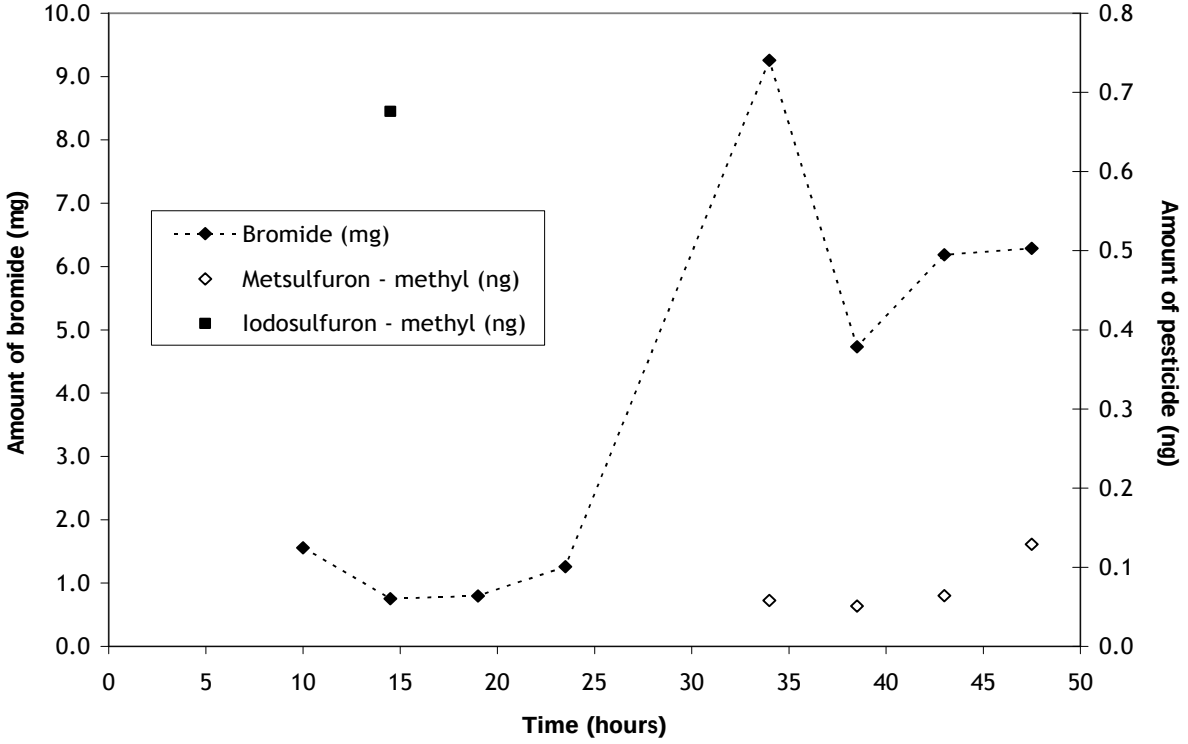

Figure 14. Leaching of bromide and iodosulfuron-methyl from soil column no. 11

Iodosulfuron-methyl was found in one of eight collected water fractions, approximately 15 hours after application, in column 11. The total amount recovered was 0.009 % of added iodosulfuron-methyl. The metabolite metsulfuron-methyl was found in the four last fractions, following the bromide front. Iodosulfuron-methyl has a DT_{50} value in soil of 1 – 5 days and the stability in water is 4 – 31 days at pH 4 – 5. Metsulfuron-methyl has a DT_{50} value in soil of 11 – 51 days and a half-life in water of 22 days at pH 5 (The e-Pesticide Manual, 2006). As mentioned earlier, the soil pH affects the degradation. As the water fractions had a pH around 4 – 5, rapid hydrolysis during the water collection cannot be excluded. Iodosulfuron-methyl mainly degrades by microbial degradation and the main degradation products are metsulfuron-methyl (88 %) and INA4098 (33 %). According to the e-Pesticide Manual, iodosulfuron-methyl and its metabolites have almost no vertical transport in soil and that there is a minimal risk for leaching below 1 meter. This study however shows that there is a possibility that both iodosulfuron-methyl and the metabolite metsulfuron-methyl can leach below 45 cm in soil.

The most important pathways for degradation of sulfonylurea herbicides in soil are chemical hydrolysis and microbial degradation, while other dissipation processes such as volatilization and photolysis are relatively insignificant. The similarity between all the sulfonylurea herbicides tested in this soil column experiment, is that they usually have fast degradation in soil and rapidly hydrolysis in water. In addition, they are quite mobile which again may lead to leaching into groundwater. The intensity and timing of precipitation, according to application date, is very important due to the transport of pesticides in soil and the risk for leaching to deeper soil layers and to groundwater bodies (Stenrød *et. al.* 2007). The transport of pesticides from agricultural fields to groundwater bodies is mainly due to

water infiltration through soil. The process occurs through matrix flow, but in several cases large and discontinuous macropores operate as preferential flow pathways and can cause rapidly movement of pesticides through soil (Flury, 1996; Guzzella *et. al.*, 2001). In this experiment, repacked columns were used and matrix flow was probably the most important flow path since a repacked column doesn't have macropores and other preferred flow paths such as cracks and paths made by earthworms. If the main flow path is primarily matrix flow, there is an increased potential for sorption to soil particles which again may lead to reduced risk for leaching (Stenrød *et. al.*, 2007).

GUS index

The GUS score was calculated according to the following relationship: GUS = log (soil DT_{50}) x (4 – log K_{OC}). As the half-life and sorption of the sulfonylurea herbicides to the the Syverud soil is not known, the GUS index was calculated using K_{OC} and half-life values from the groundwater model MACRO_GV. The MACRO_GV model is based on the model MACRO 5.0 (Larsbo and Jarvis, 2003). The results are summarized in Table 10.

Table 10. K_{OC}, DT_{50} and GUS values for the three sulfonylurea herbicides and their metabolites. L = potential leacher, T = transient properties.

	K_{OC}	DT_{50}	GUS	Classification
Tribenuron-methyl	12.5	10.8	3.0	L
INL5296	89.4	224	4.8	L
INA4098	97.7	22	2.7	T
Amidosulfuron	42	21	3.1	L
Iodosulfuron-methyl	45	8	2.1	T
Metsulfuron-methyl	39.5	24	3.3	L

The GUS index splits the studied pesticides into two groups; leachers and "borderline" compounds (transient properties) based on sorption and persistence properties in soil. Tribenuron-methyl, INL5296, amidosulfuron and metsulfuron-methyl are classified as potential leachers, while INA4098 and iodosulfuron-methyl have transient properties. Both the metabolites INL5296 and metsulfuron-methyl have a higher GUS index than their parent compounds. For these sulfonylureas, metabolites are more persistent than their parent compound and thereby represent a higher risk for leaching. This calculation of the GUS index shows that all compounds studied in this experiment can be potential contaminants of groundwater bodies. The GUS index ranges the leachability of the compounds in the following order: INL5296 > Metsulfuron-methyl > Amidosulfuron > Tribenuron-methyl > INA4098 > Iodosulfuron-methyl.

Conclusions

Tribenuron-methyl, amidosulfuron, iodosulfuron-methyl and the metabolite metsulfuron-methyl were found in leached water from columns where these pesticides were applied. The results for tribenuron-methyl may be questioned, as tribenuron-methyl and the metabolite INL5296 were detected in water fractions descended from a column were the pesticide was not added. The reason for this is probably due to the fact that the soil may have tribenuron-methyl residues from spraying the previous year (2006). The blank soil should therefore be analysed for residues. The degradation rate of the pesticides in the Syverud soil was not examined in this study. The tested pesticides obtained low recoveries compared with the initial amount applied (the total amount recovered was 0.005 % of added tribenuron-methyl, 0.009 % of added amidosulfuron and 0.009 % of added iodosulfuron-methyl). The actual total concentrations of herbicides in the drainage water was 0.0016 µg/L tribenuron-methyl, 0.018 µg/L amidosulfuron, 0.0033 µg/L iodosulfuron-methyl (0.0047 µg/L including metsulfuron-methyl). The low recoveries can be explained with fast degradation in soil, rapid hydrolysis in water and the water infiltration through soil.

GUS index calculations classify the leachability of the compounds in the following order: INL5296 > Metsulfuron-methyl > Amidosulfuron > Tribenuron-methyl > INA4098 > Iodosulfuron-methyl. Our results show that iodosulfuron-methyl may be more prone to leaching in the Syverud soil than predicted by the GUS index. The GUS index splits the studied pesticides into two groups: leachers and "borderline" compounds based on sorption and persistence properties in soil. Tribenuron-methyl, amidosulfuron and metsulfuron-methyl are classified as potential leachers, whereas iodosulfuron-methyl has transient properties. This calculation shows that all the sulfonylurea herbicides in this experiment can be potential contaminants of groundwater bodies.

Leaching in repacked columns cannot be directly compared to the situation in agricultural fields, as the microbial activity in sieved soil may differ from agricultural field soils and preferential flow paths are diminished or non-existent in repacked columns. The results should preferentially be used together with results from field experiments in order to decide whether the sulfonylurea herbicides have a high potential for leaching or not. However, the observed leaching below 45 cm of all three (four) sulfonylurea herbicides during the column study, may indicate that the risk of leaching may be even higher in the field, as field soil macropores can mitigate a more rapid flow of water and mobile pesticides than in repacked columns.

7. Sulfonylurea herbicides in surface and drainage water from a barley field

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Introduction

Sulfonylurea herbicides are widely used all around the world for the control of weeds. As the sulfonylurea herbicides are highly active at low application rate, it is a great analytical challenge to investigate the loss and the risk for sulfonylureas to reach non target sites such as brooks and rivers. Sulfonylurea herbicides are weak acids that generally exist in an ionic form in soils, and can be quite mobile depending on pH and content of organic carbon. Concerning their extensive areal use, a thorough understanding of the sulfonylureas persistence and transport behaviour is, therefore, highly needed.

Experiments carried out at a plot scale have several advantages with respect to runoff studies of pesticides. Small scale experiments can easily be controlled. The plots are subject to natural climatic conditions, and by collecting all runoff water at the edge of the field, a mass balance approach concerning loss of pesticides can be attained.

Major objectives of the study were:

To obtain data on sulfonylurea herbicides in surface and drainage water from well controlled field experiments. Are we able to trace sulfonylurea herbicides in the water that leaves the plot?

To investigate the transport pathway of sulfonylureas. To what extent does drainage water carry sulfonylurea herbicides compared to surface runoff? Will sulfonylureas dissipate during the soil infiltration process?

To detect degradation products of tribenuron-methyl. Are we able to detect degradation products of pesticides applied at very low rates, and how persistent are the degradation products?

To quantify the loss of the sulfonylurea herbicides: amidosulfuron and tribenuron-methyl through surface and drainage flow compared to the amount applied

Material and methods

The experimental site:

Syverud is located at the eastern side of the lake Årungen, approximately 4 km from the climate station Ås (Søråsjordet) and 35 km south of Oslo. The experimental plots are 27 m long and 7 m wide with a slope of 13%. The soil has a relatively high content of nutrients, is rich in the coarser size fraction and has a high aggregate stability and infiltration capacity. The erodibility of the soil is low, so the soil loss is relatively low except for extreme runoff events promoting high surface runoff. Grain size distributions at 0-20 cm are as followed: 25 % sand, 49 % silt and 26 % clay, with minor differences between the upper (0-20 cm) and lower layer (20- 40 cm) (Table 12). Total carbon is higher in the upper (3 %) compared to the lower layer (2 %) (Table 11). The soil at Syverud was tile drained more than 40 years ago, so physical conditions are well stabilized.

Table 11a and b: Soil characteristics of the experimental plots at Syverud for two different layers: 0-20 cm and 20-40 cm.

a)

Depth	pH	Tot. C	Tot. N	H^+	Ca	Mg	Na	K
Cm		%	%	cmol/kg	cmol/kg	cmol/kg	cmol/kg	cmol/kg
0-20	5,4	2,9	0,24	10,17	4,28	1,37	0,03	0,51
20-40	5,4	1,9	0,16	8,50	3,83	1,30	0,03	0,30

b)

Depth	Al-oxalat	Fe-oxalat	P-oxalat	Bulk density*	Pore volume*	Drain. Pores 10 kDa*
Cm	%	%	%		%	%
0-20	0,19	0,84	0,07	1,22	54,4	16
20-40	0,16	0,79	0,06			

*From Lundekvam and Skøien, 1998

Table 12. Grain size distribution (%) at Syverud for two different layers; 0-20 cm and 20-40 cm.

Depth (cm)	Sand (mm)			Silt (mm)			Clay (mm)
	2-0.6	0.6-2	0.2-0.6	0.06-0.02	0.022-0.006	0.006-0.002	<0.002
0-20	4.8	9.1	11.4	16.2	18.1	14.5	25.9
20-40	4.8	9.4	11.6	16.7	17.9	14.1	25.5

Treatment of the sites:

The experimental plot that received sulfonylurea herbicides was subject to autumn ploughing and spring harrowing. Dates for the different operations are given in Table 13. Similar agricultural practices have been performed since 1990. Meadow and pasture was prevailing prior to 1990.

Pesticides and bromide were applied 7. June 2007 (Table 13). It was applied 58.2 g/ha a.i. of amidosulfuron (Gratil) and 9.1 g/ha (a.i.) of tribenuron-methyl (Express). The tracer KBr was applied at the same time as the pesticides to follow the transport of water. It was applied 100 kg KBr/ha corresponding to 67 kg Br/ha at the time of spraying, see also Appendix 7. Each spring approximately 90 kg N/ha have been applied to the fields.

Table 13. Dates for the different operations carried out in 2007 at Syverud.

Application of pesticides and Br (date)	07.06.07
Plowing (date)	16.04.07
Harrowing (date)	20.04.07/31.10.07
Sowing (date)	02.05.07

Sampling procedure:

The individual plots were separated by soil mounds at each side, and a ditch in the upper end. At the lower end of the field was a perforated pipe that collected surface runoff from the plot. Water that drained through the pipes entered a tilting bucket that recorded the amount of water. The number of tilts was recorded continuously by data loggers with 5 min. resolution. Water proportional samples were collected from surface runoff and drainage water. The depth of the drains was approximately 1 m. The sampling frequency varied from a couple of days to several weeks depending on amount of runoff. Water samples were transferred to plastic containers (PE quality), and delivered to the Bioforsk Plant Health and Plant Protection for pesticide analyses (methods described in other chapters of this report). Turbidity and bromide were measured according to a standardised method at Department of Plant- and Environmental Sciences, Norwegian University of Life Sciences.

Figure 15. The Syverud field on the day of spraying with sulfonylurea herbicides; 7. June 2007. Photo: Marit Almvik.

Results and discussion:

Climatic and hydrological conditions

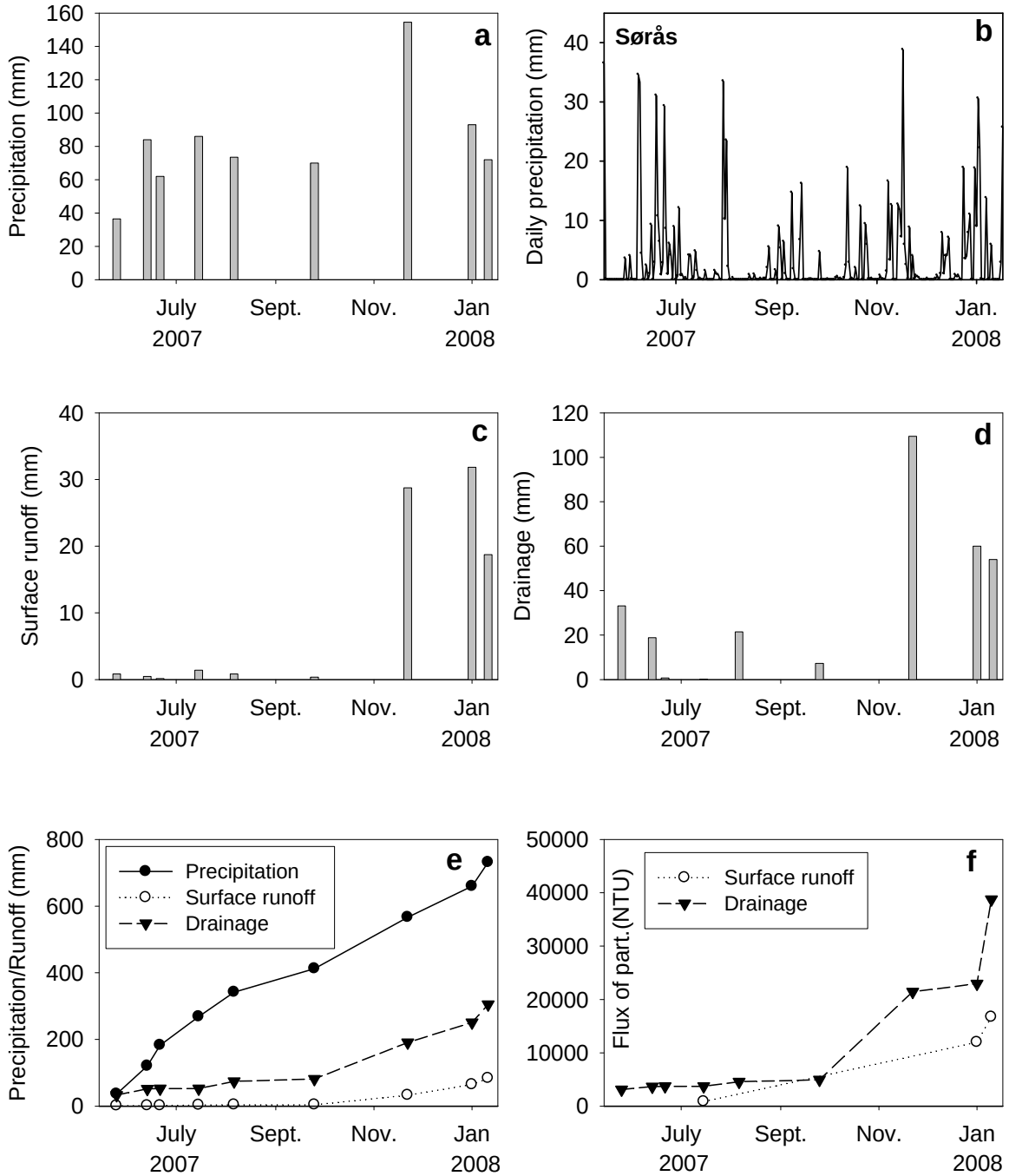


Figure 16. Important hydrological data from the period: 1. June 2007 - 31. January 2008. Accumulated precipitation (a) and water flow data (c and d) measured at time of water sampling – Syverud. Accumulated hydrological and turbidity values (an indirect measure of particle loss) for the whole period are shown in d and e. Precipitation data on a daily basis (Ås) is shown in b.

The experimental period was wetter than normal. From 1.June 2007 until 31.January 2008 the Ås area received 886 mm precipitation, which is 223 mm more than the normal value of 666 mm (Table 14)

Table 14. Average monthly precipitation (mm) and temperature (°C) at Ås (Søråsjordet) during the period 1. May 2007- 31. January 2008. Monthly average values during the normal period (1960-1990) are given in cursive.

Year	2007								2008
Month	M	J	J	A	S	O	N	D	J
Prec.	76,1	129,2	142,2	75,2	71,7	30,7	96,6	87,9	176,4
(mm)	*60*	*68*	*81*	*83*	*90*	*100*	*79*	*53*	*49*
Temp.	10,1	16,1	15,4	15,7	10,7	6,7	1,0	-1,3	1,1
(°C)	*10,3*	*14,8*	*16,1*	*14,9*	*10,6*	*6,2*	*0,4*	*-3,4*	*-4,8*

Especially, the summer months June and July received much higher amounts of precipitation than normal, with 271 mm compared to 149 mm for the normal period (1960-1990). Several years, high evapotranspiration during warm summer months leaves very low amounts of water available for runoff through the surface or drainage system. During the summer of 2007, however, there was a surplus of water that left the fields (Fig.16c and d). In this sense, the climatic conditions of the summer 2007 favoured runoff of pesticides. While August through October were drier than normal, November, December and especially January 2008 received high amount of precipitation, contributing to high water flow. A monthly precipitation of 176.4 mm in January against 49 mm (normal) was a record for the meteorological station at Ås (FAGKLIM 2008). In addition, higher temperatures than normal were observed during the winter months, with periods above 0°C, during December and January promote high runoff of water.

The soil at Syverud has a high infiltration capacity, so surface runoff during the summer months is generally low. Even for a rainy summer like 2007, the major part of the water flow occurred through the drainage system. Enough water for chemical analyses had passed the drains already at 27.June, 20 days after application of pesticides.

As expected, the concentration of particles (measured as turbidity) was generally higher in the surface runoff than for the drainage water. Total fluxes of particles were, however, higher through the drainage water caused by higher transport of water through the drains. Earlier investigations have shown that the particle sizes differ between the two transport pathways, where the smaller size fractions are more important for the drainage water compared to the surface runoff where the coarser fractions are more dominating. With respect to the transport of sulfonylurea herbicides, particle transport is probably of minor importance as their sorption to soil particles is low.

Transport of sulfonylurea herbicides through surface and drainage water

Both amidosulfuron and tribenuron-methyl were measured in surface runoff and drainage water at concentrations well above the detection limit (0.0001 µg/L). Peak concentrations were measured at the first significant rainfall event (27.June) inducing drainage water after application of the pesticides (Figure 17). The time between application and collection of drainage water samples was only 20 days, which might explain the relatively high values for the sulfonylurea herbicides – 0.31 and 0.08 µg/l of amidosulfuron and tribenuron-methyl, respectively. At the next sampling period, 5. July (day 28 after spraying), the concentration of amidosulfuron and tribenuron-methyl were much lower, being reduced to 0.03 and 0.01 µg/l. Although at low concentrations, the sulfonylurea herbicides were still present in the drainage water for quite a while. At 9.October (day 124) the concentration of tribenuron-methyl was 0.002 µg/l and at 6.December (day 182), the concentration of amidosulfuron was 0.004 µg/l. Thus, the sulfonylurea herbicides were not completely degraded half a year after application (see also Appendix 6). Much less water left the field as surface runoff, and there was only one sampling event during the summer months. At 29.July (day 52), 0.2 µg/l and 0.001 µg/l of amidosulfuron and tribenuron-methyl were measured in the surface runoff. Neglectible amounts of both pesticides were measured in surface runoff for the next two sampling events in January (day 224 and 231).

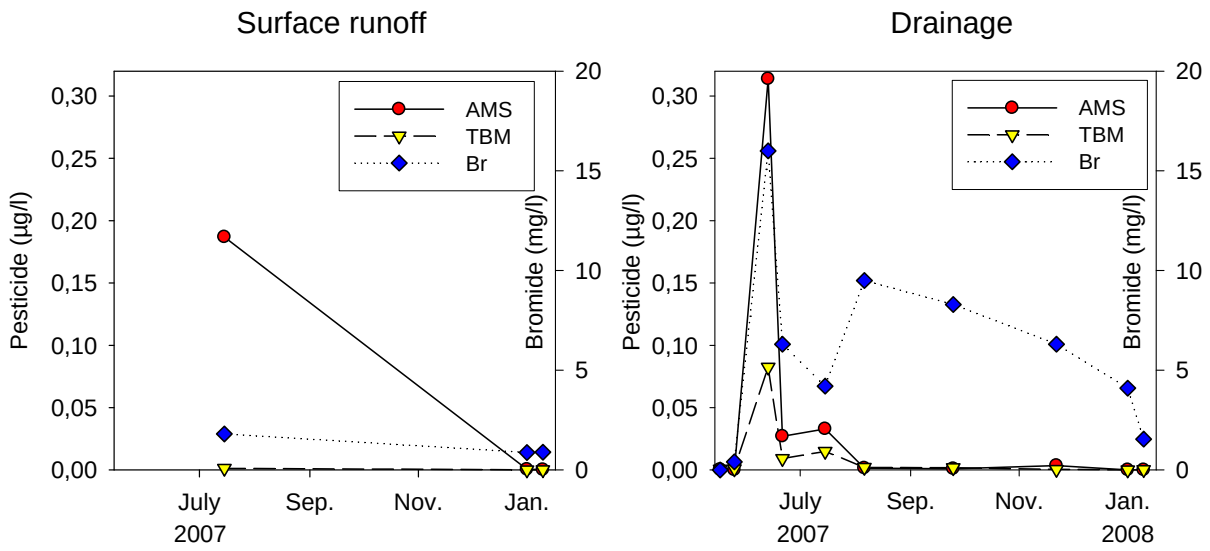


Figure 17. Presence of amidosulfuron (AMS), tribenuronmetyl (TBM) and bromide (Br) in surface runoff and drainage water at Syverud during the period 1. June 2007 – 31. January 2008. See Appendix 6 for raw data.

For the drainage water, bromide followed the pesticide peak during the first part of the period, with a high sharp peak that rapidly decreased. During a drier period, from the end of July, a second peak in the bromide concentration appeared. High evapotranspiration and minor input of water from precipitation might be the reason for the increase in bromide concentration. After the second bromide peak at 20.August (day 75), the concentration slowly decreased. Only small amounts of bromide followed the surface runoff, reflecting that the major part of the water was infiltrating the soil.

Degradation products of tribenuron-methyl

Degradation products of tribenuron-methyl were measured both in surface runoff and in drainage water at Syverud. For the drainage water, peak concentrations of the degradation products were measured at the first significant runoff episodes after application, just as for the mother compound tribenuron-methyl (Figure 18). Gradual increase in the proportion of the degradation products with time was observed, where the concentration of the degradation products INL5296 and INA4098 were 4-10 times higher than tribenuron-methyl in December and January. The numbers are, however, rather low, and care should be taken in the interpretation of the results. For the surface runoff another pattern appeared. Here, the degradation products always were at a higher concentration than for the mother compound. At the first sampling event at the end of July (day 52), the concentrations of all degradation products were more than twice as high as for tribenuron-methyl. This may indicate that the degradation process proceeds faster in the upper parts of the soils. For the last sampling event in January, the highest concentration of any degradation products was measured, where INA4098 showed the value 0.01 µg/l. Higher degradation in the upper part of the soil is consistent with higher number of microorganisms, which is shown to be essential for the degradation of sulfonylurea herbicides (Andersen *et al.* 2001). Hydrolysis is another important pathway for the degradation of sulfonylurea herbicides, where decreasing pH may enhance the degradation. The surface runoff had lower pH values (5.2-6.3) than the drainage water (6.4-7.5), which probably contributed further to higher degradation in the upper part of the soil profile.

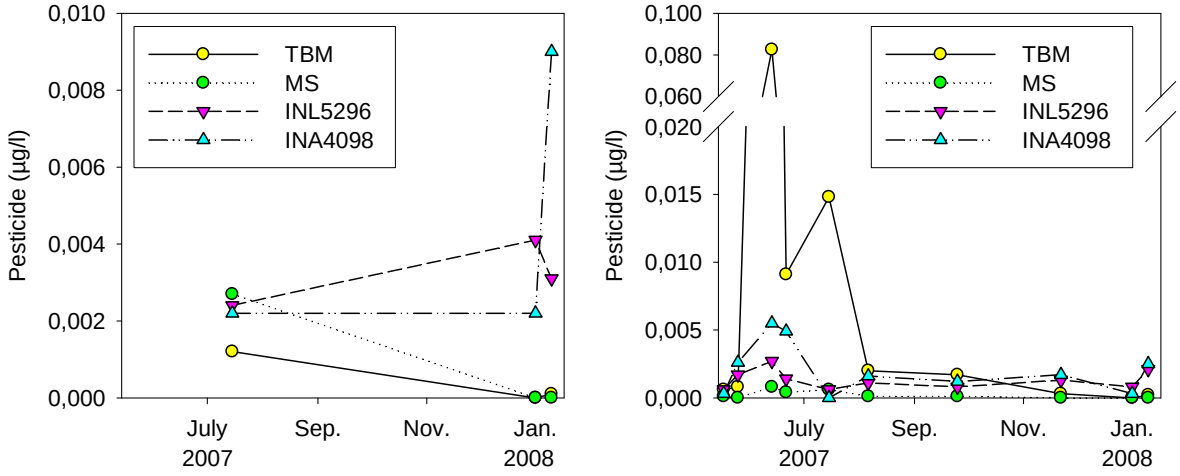


Figure 18. Concentration of tribenuronmetyl (TBM) and the degradation products metsulfuron (MS), INL5296 and INA4098 in surface runoff and drainage water at Syverud during the period 1. June 2007 – 31. January 2008.

Surprisingly, low amounts of metsulfuron-methyl occurred in both surface and drainage water from the field. The highest amounts were observed in the surface runoff. The field was not previously sprayed with metsulfuron-methyl and thus - although not reported elsewhere - we suggest that metsulfuron-methyl either is a degradation product of tribenuron-methyl or a by-product from the synthesis of tribenuron-methyl. This is supported by the fact that low amounts of metsulfuron-methyl was detected in pure tribenuron-methyl standard (purchased from Dr. Ehrenstorfer) and in the formulated product Express.

Loss of pesticides in quantity terms

Table 15. Waterflow (mm), loss (g/ha) and average concentration (µg/l) of the pesticides amidosulfuron and tribenuron-methyl during the period 01.06.2007 - 31.01.2008 at Syverud.

Type	Volume (mm)	Loss		Concentration (avg)	
		Amidosulfuron (g/ha)	Tribenuron-methyl (g/ha)	Amidosulfuron (µg/l)	Tribenuron-methyl (µg/l)
Surface	83	0.006	0.0001	0.007	0.000
Drainage	305	0.064	0.0169	0.021	0.006
Sum	388	0.070	0.0170	0.018	0.004

Largest loss of pesticides occurred through drainage water as the major proportion of the water infiltrated the soil before it left the fields. In addition, low average concentration in the surface runoff, indicate that the pesticides are mobile and are relatively quickly transferred to deeper parts of the soil. As amidosulfuron was applied at a higher amount than tribenuron-methyl, the loss in g/ha was also higher for this pesticide. Although detectable amounts of both pesticides were measured for several months after application, the flow event just after application was responsible for the major loss (Figure 19).

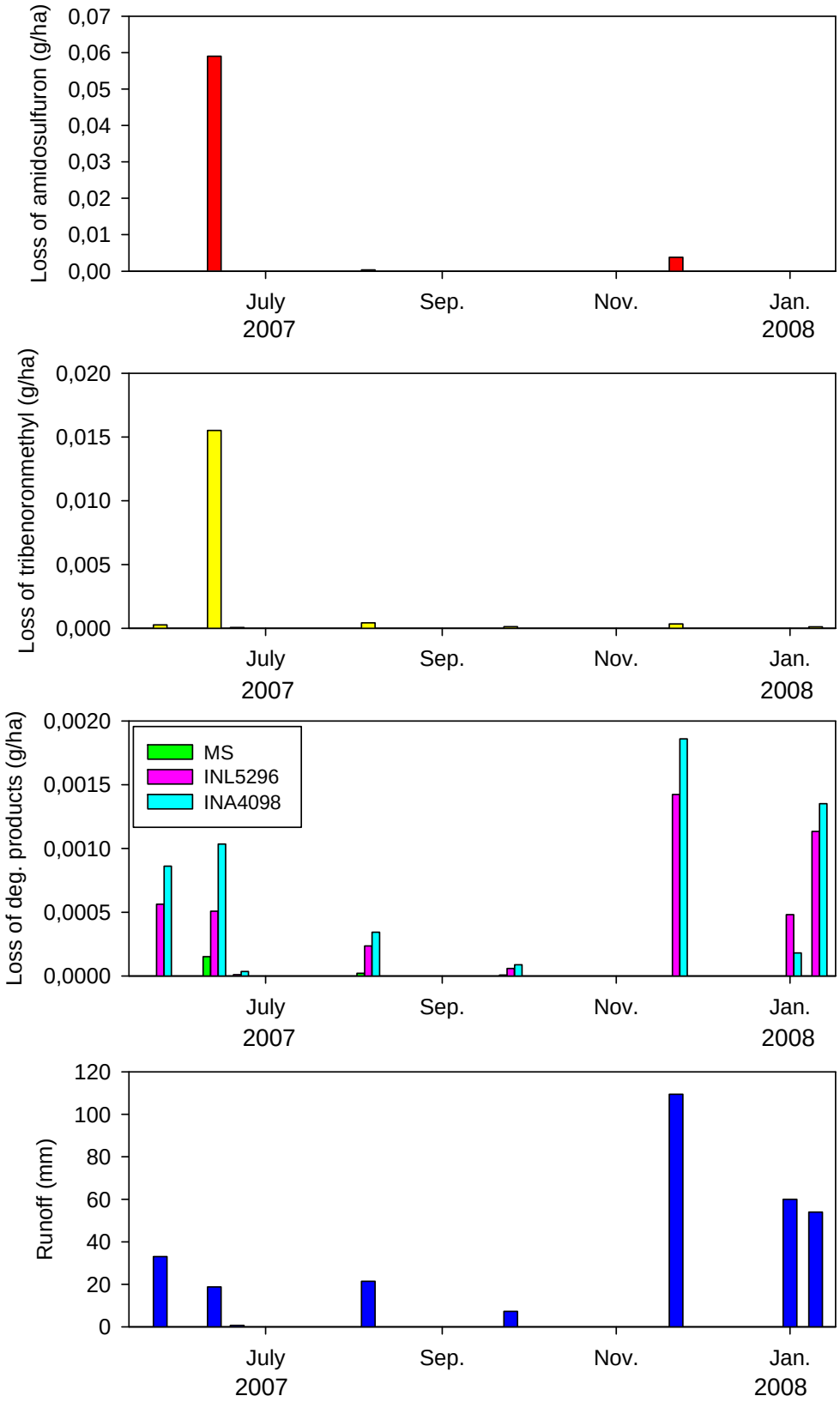


Figure 19. Loss of amidosulfuron, tribenuron-methyl, and the degradation products: metsulfuron (MS), INL5296 and INA4098 in drainage water. All results for the pesticides are given in g/ha. Runoff of drainage water is given in mm.

Table 16. Loss of amidosulfuron and tribenuron-methyl in percent of the amount applied, during the period 01.06.2007 – 31.01.2008. The plots received 58.2 g/ha of amidosulfuron (a.i.) and 9.1 g/ha of tribenuron-methyl (a.i.). DP = degradation products of tribenuron-methyl.

Water flow	Amidosulfuron (%)	Tribenuron-methyl (%)	Tribenuron-methyl + DP (%)
Surface	0.01	0.001	0.071
Drainage	0.11	0.185	0.299
Sum	0.12	0.186	0.370

Based on percentage values, the loss of tribenuron-methyl (app. 0.2 %) was somewhat higher than for amidosulfuron (app. 0.1 %). If the loss of degradation products of tribenuron-methyl also are taken into account, the loss adds up to approximately 0.4 %. The importance of the degradation products increased with time, being most important during runoff episodes during the winter months of December and January. Hence, winter climate might be of great importance for the runoff of degradation products, where mild winters may enhance the transport. However, greatest concern has to be given to flow episodes close to application. Amidosulfuron attained values higher than the drinking water limit both in surface (0.2 µg/l) and drainage water (0.3 µg/l) during the summer of 2007. With respect to tribenuron-methyl, the peak concentration was also close to the drinking water limit and the PNEC (Predicted No-Effect Concentration) which both are set to 0.1 µg/l.

It is also noticeable that highly active pesticides applied at low amounts are able to infiltrate the soil and leave the fields through the drains for a soil such as Syverud, with relatively high amount of organic carbon and high aggregate stability. This may indicate that for soils with high infiltration capacity, sulfonylurea herbicides can be transported into deeper parts of the soil, where the degradation passes more slowly compared to higher up in the soil profile.

Conclusions:

The sulfonylurea herbicides amidosulfuron and tribenuron-methyl were measured in both surface runoff and drainage water at concentration well above the detection limit. Peak concentrations of 0.31 µg/l amidosulfuron and 0.08µg/l tribenuron-methyl were measured at the first significant flow event after application of pesticides.

The sulfonylurea herbicides infiltrated rapidly the soil and left the fields through the drainage system only three weeks after application.

Transport of sulfonylurea herbicides through the drainage water was far more important than surface runoff, as the majority of water left the fields through the drains, the investigated period received high amount of precipitation and the sulfonylureas show high mobility in soils.

Degradation products of tribenuron-methyl were measured both in surface runoff and drainage water. For the drainage water, peak concentrations of the degradation products were measured at the first significant leaching period after application, just as for the mother compound tribenuron-methyl. Gradual increase in the proportion of degradation products compared to the mother compound occurred with time. For the surface runoff another pattern appeared. Here the degradation products always were at a higher concentration than for the mother compound. The degradation apparently proceeds faster in the uppers soil layer, both as a result of higher microbial degradation and more efficient hydrolyses caused by lower pH in the upper compared to deeper layers of the soil profile. A climate with warmer winters may results in higher runoff of degradation products compared to winters where the soil is frozen.

Approximately 0.1 % of amidosulfuron and 0.2 % of tribenuron-methyl were lost through water flow, were drainage water was far the most important. If degradation products also are taken into account, the loss of tribenuron-methyl adds up to 0.4 %.

An important knowledge from the study is that the sulfonylurea herbicides have a relatively high ability to infiltrate soil, to deeper soil layers where the degradation proceeds slower and the mother compound can persist for a longer period of time.

8. Conclusions

Lack of sufficiently sensitive analytical methods has been a major obstacle in gaining information on the leaching of the sulfonylurea herbicides. The LC-MS/MS method developed during this project has a very good sensitivity for the selected sulfonylurea herbicides and metabolites. The SPE method gave a 400-fold preconcentration of the water samples with good recoveries for the herbicides, although not optimal for the more polar metabolites. An alternative SPE method is proposed for the metabolites.

The GUS index calculation showed that all the sulfonylurea herbicides in this experiment can be potential contaminants of groundwater bodies. The soil column study showed that the herbicides were able to leach through 45 cm packed soil columns, with the highest relative concentrations for amidosulfuron and iodosulfuron-methyl, followed by tribenuron-methyl. The total amounts leached were very low (0.018, 0.0047 and 0.0016 µg/l, respectively). Iodosulfuron-methyl was seen to leach both as the mother compound and as the metabolite metsulfuron-methyl, whereas tribenuron-methyl was only recovered as the mother compound, reflecting the different half-lives of these herbicides in soil. The results for tribenuron-methyl may be questioned, as tribenuron-methyl and the metabolite INL5296 were detected in water fractions descended from a column were the pesticide was not added. The blank soil should be analysed for residues.

Leaching of amidosulfuron, tribenuron-methyl and the degradation products metsulfuron-methyl, INL5296 and INA4098 to tile drains 1 m deep was observed in the Syverud field study. The amounts recovered were higher by a factor of 40 for tribenuron-methyl and 11 for amidosulfuron as compared to the soil column study, showing the importance of macropores and preferential flow patterns for the leaching of these very mobile herbicides in the field.

Although at low concentrations, the sulfonylurea herbicides were still present in the drainage water in December. Thus, the sulfonylurea herbicides were not completely degraded half a year after application. Due to low microbial activities in the subsoil, tribenuron-methyl was seen to leach predominantly intact into the drains, whereas in the surface runoff, a rapid degradation of the herbicide could be observed by the dominating and increasing presence of degradation products. A climate with warmer winters may results in higher runoff and leaching of degradation products compared to winters where the soil is frozen. However, greatest concern has to be given to transport episodes close to application, when the herbicide concentrations are seen to approach or even exceed drinking water limits and PNEC values.

Degradation products of amidosulfuron were not included in the field study, as it was difficult to establish an analytical method in the given time-frame. All of the major degradation products of amidosulfuron are hydroxylated and hence very mobile. Sulfonylurea herbicides produce an array of different degradation products with varying mobility and persistence. INL5296 and INA4098 are amongst the more persistent degradation products, but their toxicity is low. The sulphonamide degradates (“left ring” e.g. saccharin) however, are generally much more mobile and also more toxic. There is little information about the degradation, fate and toxicity of the metabolites of sulfonylurea herbicides in the field. This is an important issue, as we have seen that in the field, both amidosulfuron and tribenuron-methyl is readily transported and subsequently degraded. Similar studies need also to be performed with the other approved sulfonylurea herbicides in Norway.

9. References

Andersen, SM, Hertz, PB, Holst, T, Bossi, R and Jacobsen, CS, 2001. Minseralisation studies of 14C-labelled metsulfuron-methyl, tribenuron-methyl, chlorsulfuron and thifensulfuron-methyl in one Danish soil and groundwater sediment profile. Chemosphere 45: 775-782.

Barrett, MR, 1996. Chapter 16, The Environmental Impact of Pesticide Degradates in Groundwater. In *Herbicide Metabolites in Surface Water and Groundwater*, M. T. Meyer and E. M. Thurman, eds. (Washington, D.C.: American Chemical Society), pp. 200-225.

Beyer, EM., Duffy, MJ, Hay, JV and Schlueter, DD, 1988. Sulfonylureas. p.117-189 in Herbicides: Chemistry, Degradation, and Mode of Action, Vol. 3. Kearney, PC. and DD Kaufman, eds. Marcel Dekker Inc., New York.

Bossi, R, Koppen, B and Spliid, NH, 1998. Analysis of sulfonylurea herbicides in soil water at sub-part-per-billion levels by electrospray negative ionization mass spectrometry followed by confirmatory tandem mass spectrometry. Journal of AOAC Int. 81(4): 775-784.

Bossi, R, Vejrup, K and Jacobsen CS, 1999. Determination of sulfonylurea degradation products in soil by liquid chromatography-ultraviolet detection followed by confirmatory liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 855: 575-582.

Branham, B, Milnert, E and Rieke, P, 1995. Potential groundwater contamination from pesticides and fertilizers used on golf courses. USGA Green Section Record, vol. 31 (1): 33-37.

Brown, HM. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci. 29: 263-281.

FAGKLIM 2008: http://www.umb.no/imt/fagklim

Flury, M, 1996. Experimental evidence of transport of pesticides through field soils – a review. Journal of Environmental Quality 25: 25-45.

Font, N, Hernandez, F, Hogendoorn, EA, Baumann, RA and van Zoonen, P, 1998. Microwave-assisted solvent extraction and reversed-phase liquid chromatography-UV detection for screening soils for sulfonylurea herbicides. Journal of Chromatography A 798: 179-176.

Fontanals, N, Marcé, RM and Borrull, F, 2007. New materials in sorptive extraction techniques for polar compounds. Journal of Chromatography A, 1152: 14-31.

Galletti, GC, Bonetti, A and Dinelli, G, 1995. High-performance liquid chromatographic determination of sulfonylureas in soil and water. Journal of Chromatography A 692: 27-37.

Gustafson, DI, 1989. Groundwater ubiquity score: a simple method for assessing pesticide leachability. Environmental Toxicology and Chemistry 8, 339-357.

Guzzella, L, Pozzoni, F, Rullo, S, Giuliano, G, 2001. Mobility of herbicides used for weed control in maize in lysimeter experiments. In: Walker A. (Ed), Proceedings of Pesticide Behaviour in Soils and Water, Brighton UK, November 2001, 101-106.

http://www.mattilsynet.no/planter/plantevernmidler/statistikk

http://www.strataspe.com/pr_chem_stratax.asp

Kjær, J, Olsen, P, Barlebo, HC, Henriksen, T, Plauborg, F, Grant, R, Nygaard, P, Gudmundsson, L and Rosenbom, A, 2007. The Danish Pesticide Leaching Assessment Programme. Monitoring results May 1999-June 2006. ISBN 978-7871-197-7. Geological Survey of Denmark and Greenland. Available at www.pesticidvarsling.dk.

Kreuger, J, 1998. Pesticides in stream water within an agricultural catchment in southern Sweden, 1990-1996. The Science of the Total Environment 216, pp. 227-251.

Laganá, A, Fago, G, Matino, A and Penazzi, VM, 2000. Liquid chromatography mass spectrometry tandem for multiresidue determination of selected post-emergence herbicides after soil column extraction. Analytica Chimica Acta 415: 41-56.

Larsbo, M & Jarvis, NJ, 2003. MACRO 5.0. A model of water flow and solute transport in macroporous soil. Technical description. Studies in the Biogeophysical Environment, Emergo 2003:6, Department of Soil Sciences, SLU, Uppsala, Sweden, 48 pp.

Ludvigsen, GH and Lode, O, 2008. Oversikt over påviste pesticider I perioden 1995-2006. Resultater fra JOVA: Jord- og vannovervåking i landbruket i Norge. Bioforsk Rapport vol. 3, nr. ?, pp. 55-58.

Lundekvam, H and Skøien, S, 1998. Soil erosion in Norway. An overview of measurements from soil loss plots. Soil Use Manage 14: 84-89.

Netland, J and Wærnhus, K, 2007. Sulfonylurea resistance found in several weed species in Norway. 14th EWRS Symposium, Hamar 18-21 June 2007, p. 156.

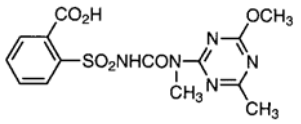
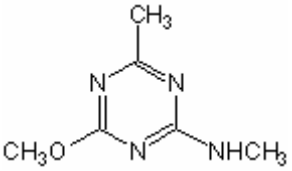
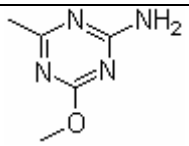
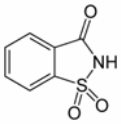
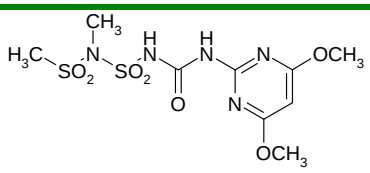
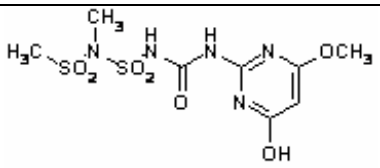
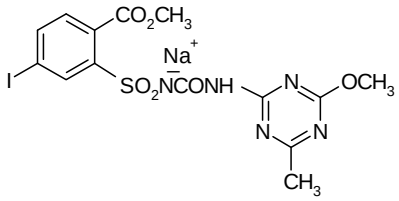
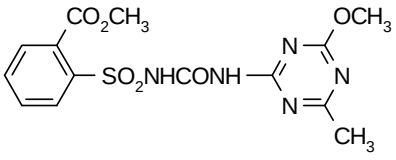
OECD Guidelines for the testing of chemicals, 2004. Leaching in soil columns.
Perreau, F, Bados, P, Kerhoas, L, Nélieu, s and Einhorn, J, 2007. Trace analysis of sulfonylurea herbicides and their metabolites in water using a combination of off-line or on-line solid-phase extraction and liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem 388: 1265-1273.
Polati, S, Bottaro, M, Frascarolo, P, Gosetti, F, Gianotti, V and Genaro, MC, 2006. HPLC-UV and HPLC-MS^n multiresidue determination of amidosulfuron, azimsulfuron, nicosulfuron, rimsulfuron, thifensulfuron methyl, tribenuron methyl and azoxystrobin in surface waters. Analytica Chmica Acta 579: 146-151.
Reemtsma, T and Quintana, JB, 2006. Analytical methods for polar pollutants. In: Organic Pollutants in the Water Cycle. T. Reemtsma and M. Jekel (Eds.). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, ISBN: 3-527-31297-8.
Stenrød, M, Ludvigsen, GH, Riise, G, Lundekvam, H, Almvik, M, Tørresen, KS, Øygarden, L, 2007. En sammenstilling av norske og internasjonale forsknings- og overvåkingsresultater, samt en småskala feltstudie av avrenning av glyfosat ved ulik jordarbeiding. Bioforsk Rapport 2/145. 87 p.
The e-Pesticide Manual, 2006. British Crop Protection Council.
Waage Tveit, C, 2007. Notat om sulfonylureastoffene. Verdier hentet fra Mattilsynets vurdering i 2000 og EUs list of endpoint. 5 p.

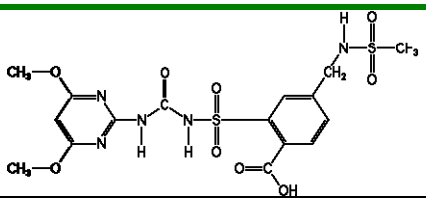
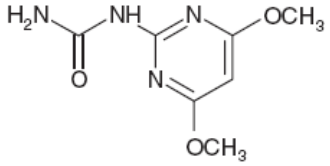
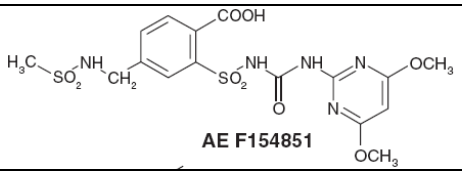
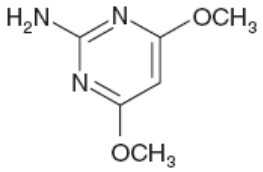
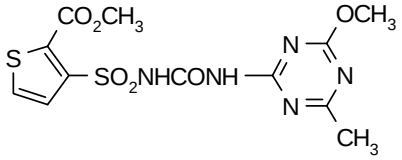
10. Appendixes

Summary of appendixes

No.	Subject
1	Overview of sulfonylurea herbicides approved in Norway (2008)
2	Description of various polymeric solid phase extraction sorbents
3	MS/MS-parameters for sulfonylurea herbicides
4	Soil profile characterisation of the Syverud soil
5	Leaching data from the lysimeter study
6	Chemical properties of water leaving the Syverud field
7	Spraying plan for the Syverud field

APPENDIX 1: Overview of sulfonylurea herbicides approved in Norway (2008)

Herbicide/metabolite	Structure	Data (e-Pesticide Manual, 2006)
Tribenuron-methyl		pK_a = 5.0 DT_{50}= 2-12 days Hydrolysis (45°C): rapid < pH7, stable pH 8-10 K_d = 0.2 – 2 Water solubility: 280 mg/l (pH6), 2.0 g/l (pH7)
INL5296 Max 90% 4- methoxy- N,6-dimethyl-1,3,5- triazin-2-amine Syn. N-methyl triazine amine		DT_{50}= 148 days in soil DT_{50}= 70-98 d. in water K_f = 0.9- 3.2 Hydrolytically stable (pH5)
INA4098 Max 8% 4-methoxy-6-methyl-1,3,5-triazin-2-amin Syn. Triazine amine		DT_{50}= 22-240 days K_f = 0.1-0.7 pK_a = 3
IN00581 Saccharin Max 47%		DT_{50} = 31-198 days K_{oc} = 6-11 Hydrolytically stable (pH5)
Amidosulfuron 1-(4,6-dimethoxypyrimidine-2-yl)-3-mesyl(methyl)-sulfonylurea Syn. Hoe 075032 AE F075032		pK_a = 3.58 DT_{50} = 3-29 days Hydrolysis (25°C): DT_{50} = 34 d (pH5), 365 d (pH7) K_{oc} = 2.6 - 82 Watersol.: 9 mg/l (pH6), 13 g/l (pH 10)
AE F101630 Max 50% 3-(4-hydroxy-6-methoxypyrimidin-2-yl)-1-(N-methyl-N-methylsulfonyl-aminosulfonyl)-urea Syn.: Hoe 101630		DT_{50} = 3-15 dager K_{oc} = 24-63
Iodosulfuron-methyl Syn. Iodosulfuron-methyl-sodium		pKa = 3.22 DT_{50} = 1-4 days K_f = 0.01-2.5 Watersol.: 160 mg/l (pH5), 25 g/l (pH7)
Metsulfuron-methyl Max 88% *of iodosulfuron-methyl*		pK_a = 3.75 DT_{50} = 11-51 days Hydrolysis (25°C): 22 d (pH5), stable pH 7 og 9. K_f = 0.05-4.9
INA4098 Max 33% *av metsulfu*	See Tribenuron-methyl	
IN-00581 Saccharin Max 32% *av metsulfuron-methyl*	See Tribenuron-methyl	

INJX909/AEF116778 Max. 33% i vann *Bis*-O-demethyl metsulfuron-methyl		
Mesosulfuron		pK_a = 4.35 DT_{50} = 6-91 days K_f = 0.2-3.7 Watersol.: 7.2 mg/l (pH5), 483 mg/l (pH7) Hydrolysis (25°C): DT_{50} 253 d (pH 7)
AEF099095 Max. 29% 4,6-dimethoxypyrimidine-2-yl-urea		DT_{50} = 48 days Koc : 141 - 226 – 1360 Kf : 2.3 - 42.8
AEF154851 Max. 16% Mesosulfuron acid		DT_{50} = 19-71 days Koc : 46 – 98 Kf : 0.8 - 3.1
AEF092944 Max. 10% 2-Amino-4,6-dimethoxypyrimidine (Aldrich 375349) CAS Number 36315-01-2		DT_{50} = 28-88 days Koc : 89 – 917 Kf : 2.5 - 16.5
Thifensulfuron-methyl		pK_a = 4.0 DT_{50} = 2-6 days Hydrolysis (25°C): DT_{50} = 38t (pH5), 250t (pH7) Watersol.: 224 mg/l K_f = 0.08-1.4
INA4098 Max. 30%	Se forrige side	
INW8268 Max. 27% Thiofene sulfonamide		DT_{50} = 10-97 days Koc : 3-4
INL9225 Max. 25% Thifensulfuron acid		DT_{50} = 20-157 days Koc : 7-14, Kd = 0.06-0.89
INL9226 Max. 15% O-desmethyl thifensulfuronmethyl		DT_{50} = 3-15 days Koc : 34-199 Kd =0.5-4.7

APPENDIX 2. Description of various polymeric solid phase extraction sorbents

(from Fontanals *et al.* 2007)

The traditional PS-DVB (polystyrene-divinylbenzene) polymeric sorbent is a hydrophobic sorbent that interacts with analytes basically through Van der Waals forces and pi-pi interactions of the aromatic ring that make up the polymer structure. The higher the specific surface area of the sorbent, the larger the number of pi-pi sites available to interact with the analytes, thus, the extraction efficiency increases with increasing surface area. Lichrolut EN is an example of a hypercrosslinked PS-DVB sorbent, with a very high surface area (1200 m^2/g).

In order to increase the retention of polar analytes, the hydrophobic PS-DVB polymer is often modified with a polar moiety, e.g. Isolute ENV+, which is a hypercrosslinked hydroxylated PS-DVB resin and the more recent Strata X, which has a styrenic skeleton modified with a pyrrolidone group. Strata-X has been further modified with ionic groups to transform the resin into a mixed-mode ion-exchange resin, e.g. the weak cation-exchange resin Strata X-CW, which is modified with a carboxylic group (-COOH). Mixed-mode ion-exchange sorbents can interact with ionic species, but can also retain non-charged species through hydrophobic and hydrophilic interactions.

Another way of producing hydrophilic polymeric sorbents is to copolymerise monomers that contain suitable functional groups, e.g. Oasis HLB (macroporous poly(*N*-vinylpyrrolidone-divinylbenzene); PVP-DVB).

APPENDIX 3. MS/MS-parameters for the analysis of sulfonylurea herbicides

M72 LC- MS/MS

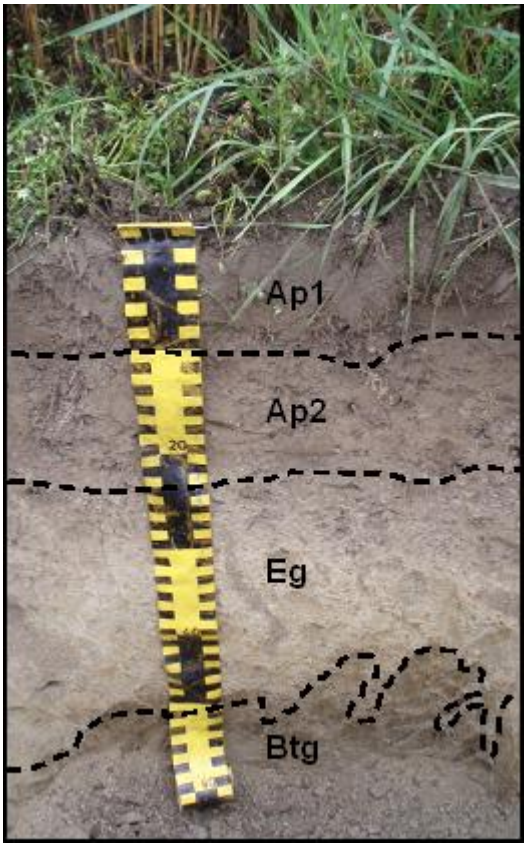
Gr. (MRM)	Compound	Formulae	R_t (min)	Molecular Weight (Môn isotopic)	Molecular Weight	pKa	Mode	No. of transitions	Parent ion	Cone Voltage	Daughter ion	Collision Energy
1	INA4098	$C_5H_8N_4O$	5,2	140,07	140,15	-	ES+	2	140,88	75	**100,06**	12
											85,07	12
	IN5296	$C_6H_{10}N_4O$	6,5	154,09	154,17	-	ES+	2	154,89	75	**114,04**	16
											71,15	16
2	Metsulfuron-methyl	$C_{14}H_{15}N_5O_6S$	8,6	381,07	381,37	3,3	ES+	2	381,93	65	**167,04**	12
											141,04	22
	Amidosulfuron	$C_9H_{15}N_5O_7S_2$	9,0	369,04	369,38	3,6	ES+	2	369,93	37	**261,02**	12
											217,97	28
	Tribenuron-methyl	$C_{15}H_{17}N_5O_6S$	9,1	395,09	395,39	5,0	ES+	2	395,97	65	**181,04**	20
											155,03	16
	Iodosulfuron-methyl	$C_{14}H_{13}IN_5O_6S$	9,4	505,96	506,26	-	ES+	2	507,32	58	**167,02**	28
											141,06	30
	Metsulfuron-methyl-D3 (IS)	$C_{14}H_{15}N_5O_6S$	8,6	384,07		-	ES+	1	385,04	65	**170,04**	22

APPENDIX 4. Soil profile characterisation of the Syverud soil

Soil characteristics of the experimental plots at Syverud achieved from The Norwegian Forest and Landscape Institute.

Sjikt	Tykkelse cm	Frasikt %	Kornstørrelsesfordeling (%)								%		
			2-0,6 mm	0,6-0,2 mm	0,2-0,1 mm	0,1-0,06 mm	0,06-0,02 mm	0,02-0,006 mm	0,006-0,002 mm	<0,002 mm	Sand	Silt	Leir
Ap1	0 - 10	6	7,23	8,47	5,48	4,87	14,3	17,9	14,8	27	26	47	27
Ap2	10 - 22	9	6,62	8,28	5,5	4,99	14,5	17,8	15,3	27,1	25	48	27
Eg	22 - 48	9	6,7	5,72	5,14	7,93	23,6	20,4	13	17,6	25	57	18
Btg	50 - 70	10	3,14	4,16	3,9	6,02	19,7	18,2	14,7	30,1	17	53	30
Cg	70+	13	2,43	3,53	3,3	3,88	14,2	17,4	16,7	38,6	13	48	39

Sjikt	Tykkelse	pH	Tot C	Tot N	Ombyttb. Kationer (meq/100g)					CEC	BM	Tørrst
	cm	H_2O	%	%	H	K	Na	Mg	Ca	meq/100g	%	%
Ap1	0 - 10	5,45	3,1	0,29	10,3	0,41	0,058	1,38	4,37	16,5	38	98
Ap2	10 - 22	5,47	2,9	0,28	9,5	0,37	0,057	1,46	4,69	16,1	41	98,1
Eg	22 - 48	5,59	0,4	0,05	3,9	0,13	0,039	0,74	1,93	6,7	42	99,2
Btg	50 - 70	6,00	0,3	0,05	3,4	0,22	0,076	2,28	6,33	12,3	72	98,7
Cg	70+	6,67			2,9	0,27	0,117	3,36	9,03	15,7	82	98,4



Soil profile characterisation of the Syverud soil. Photo: Eivind Solbakken.

APPENDIX 5. Leaching data from the lysimeter study

Column	Fraction (hours)	Volume (ml)	Br (mg)	INA4098 Conc. (µg/ml)	INA4098 Tot. amount (ng)	Metsulfuron-methyl Conc. (µg/ml)	Metsulfuron-methyl Tot. amount (ng)	Amidosulfuron Conc. (µg/ml)	Amidosulfuron Tot. amount (ng)	Tribenuron-methyl Conc. (µg/ml)	Tribenuron-methyl Tot. amount (ng)	Iodosulfuron-methyl Conc. (µg/ml)	Iodosulfuron-methyl Tot. amount (ng)
3 (added tribenuron methyl)	10	430	1.9										
	14.5	214	0.8										
	19	194	0.9							0.0002	0.125		
	23.5	196	1.9							0.0002	0.125		
	34	480	11.0										
	38.5	194	5.3										
	43	198	5.8							0.0001	0.062		
	47.5	176	5.1										
8 (added amidosulfuron)	10	410	1.7	0.0001	0.100								
	14.5	204	0.7	0.0002	0.124					0.0005	0.307		
	19	186	0.8	0.0001	0.063					0.0003	0.190		
	23.5	190	2.1	0.0005	0.315					0.0018	1.132		
	34	460	16.7	0.0001	0.114								
	38.5	187	8.0	0.0001	0.063								
	43	192	7.1	0.0001	0.063								
	47.5	184	6.1	0.0001	0.064								
9 (added amidosulfuron)	10	400	1.6										
	14.5	200	0.7										
	19	182	0.8										
	23.5	186	1.2					0.0006	0.369				
	34	460	3.8					0.0006	0.754				
	38.5	185	2.3					0.0011	0.680				
	43	190	2.9					0.0017	1.072				
	47.5	183	3.8					0.0018	1.147				
11 (added iodosulfuron methyl)	10	405	1.6										
	14.5	204	0.8									0.0011	0.676
	19	190	0.8										
	23.5	194	1.3										
	34	470	9.3			0.00005	0.058						
	38.5	190	4.7			0.00008	0.051						
	43	197	6.2			0.00010	0.064						
	47.5	186	6.3			0.00020	0.129						

APPENDIX 6. Chemical properties of water leaving the Syverud field

a) Surface runoff

Dato	Amidosulf. ($\mu g\ l^{-1}$)	Trib.met. ($\mu g\ l^{-1}$)	Met.sulf. ($\mu g\ l^{-1}$)	INL5296 ($\mu g\ l^{-1}$)	INA4098 ($\mu g\ l^{-1}$)	Br ($mg\ l^{-1}$)	pH	Turb. (NTU)
29.07.2007	0,1869	0,0012	0,0027	0,0024	0,0022	1,8	5,2	300
15.01.2008	0,0004	0,0000	0,0000	0,0041	0,0022	0,9	6,0	180
24.01.2008	0,0002	0,0001	0,0000	0,0031	0,0090	0,9	6,3	251

b) Drainage water

Dato	Amidosulf. ($\mu g\ l^{-1}$)	Trib.met. ($\mu g\ l^{-1}$)	Met.sulf. ($\mu g\ l^{-1}$)	INL5296 ($\mu g\ l^{-1}$)	INA4098 ($\mu g\ l^{-1}$)	Br ($mg\ l^{-1}$)	pH	Turb. (NTU)
31.05.2007	0,0003	0,0006	0,0001	0,0006	0,0003	<0,2	7,4	26
08.06.2007	0,0000	0,0008	0,0000	0,0017	0,0026	0,4	6,5	94
27.06.2007	0,3138	0,0825	0,0008	0,0027	0,0055	16	6,5	29
05.07.2007	0,0270	0,0091	0,0004	0,0014	0,0049	6,3	6,4	85
29.07.2007	0,0328	0,0148	0,0006	0,0006	0,0000	4,2	6,6	24
20.08.2007	0,0015	0,0020	0,0001	0,0011	0,0016	9,5	6,4	39
09.10.2007	0,0009	0,0017	0,0001	0,0008	0,0012	8,3	7,2	51
06.12.2007	0,0035	0,0003	0,0000	0,0013	0,0017	6,3	7,5	151
15.01.2008	0,0000	0,0000	0,0000	0,0008	0,0003	4,1	7,4	25
24.01.2008	0,0000	0,0002	0,0000	0,0021	0,0025	1,5	6,6	293

APPENDIX 7. Spraying plan for the Syverud field

Plan nr: 01/2007

1. Tidspunkt for sprøyting:
Uke 23, 7. juni 2007

2. Personale:
Forsøksleder: Marit Almvik
GLP-inspektør: Randi Bolli
Sprøyteansvarlig: Kjell Wærnhus

3. Feltene:
Syverud i Ås Kart over feltet er vedlagt

4. Tillaging av sprøyteløsninger:
Doser:
Express: ¼ tablett per dekar. 1 tablett veier 7.5 gram. Det skal da brukes 1.875 g. Express per dekar. Ved blanding med andre preparater, skal Express alltid tilsettes først i tanken.
Gratil 75WG: 8 g/dekar.
Væskemengde: 25 l/daa tankblanding, XR111002 dyser

Syverud: rute 3 og 4
Sprøyteareal: 25 m x 3 drag (2.5 m bom) = 187.5 kvm/rute
Væskeblanding: 15 kg
Oppveid mengde: Express: (1,875/25)*15=1.125g,
Gratil 75WG: (8/25)*15= 4.8 g
Teoretisk forbruk: 4.7 kg/rute x 2 = 9.4 kg

Feltet skal også sprøytes med metalaksyl, propikonazol og kaliumbromid samme dag, se sprøyteplan 02/2007.

5. Prøvetaking
Før sprøyting:
- ✓ Ta ut blanke jordprøver (utføres den 4. juni)
- ✓ Ta ut jord fra feltet; 3 stikk i hver rute; 0-60 cm.
- ✓ Mål dekningsgraden av kornet.
- ✓ Mål gjennomsnittlig høyde på kornet.
- ✓ Ta bilder av feltene.
- ✓ Hver rute deles i 4 underruter, deretter fordeles 3 petriskåler i hver underrute, totalt 12 skåler per hovedrute. Skålene merket A legges øverst, D nederst. Skålene merket ”1” legges til venstre, ”2” i midten og ”3” til høyre. Hver skål er 1.2 cm høy og fylles jevnt med overflatejord til toppen. Pass på å ikke presse jorda sammen når de overføres til skålene; tettheten av jorda skal være som den er når den ligger på overflata av feltet. Legg skålene på overflaten, oppå byggvekstene, slik at de er fritt eksponert for sprøytevæska. Tegn opp utleggingen.

Bruk bare ett sett skåler (2x12 stk.) for alle sprøytingene med lavdosemidler, propikonazol, metalaksyl og bromid (la skålene ligge til alle sprøytingene er utført).

Etter sprøyting:

- ✓ Bruk hansker og legg lokk på petriskålene og forsegl med parafilmstrimler langs skjøten.
- ✓ Ta ut ca. 50 ml sprøyteløsning fra tanken av lavdosemidlene i en plastflaske. Flaskene skal være merket med tittel, konsentrasjon, felt og dato.

6. Behandling/oppbevaring av prøver etter uttak

Emballasje

Etter prøvetaking overføres prøvene til egnet emballasje:

- ✓ Petriskålene overføres til merkede (felt, sprøytemidler og dato) plastposer.
- ✓ Jordprøvene oppbevares i plastposer

Transport/Lagring

- ✓ Prøvene bringes til laboratoriet samme dag.
- ✓ Ved ankomst laboratoriet registreres og lagres prøvene som angitt i prosedyre P0101.
- ✓ Petriskålene og jordprøvene fryses.

7. Registrering av data/forhold ved feltet under prøvetaking

Registrering av data gjøres i feltloggboken som inneholder skjemaet "SK34 Registrering av feltdata". I tillegg til de faste parametrene som står på skjemaet skal også alle variable faktorer som kan ha innflytelse på resultatet fra sprøytingen registreres. Dette kan være værforhold, fuktighet, topografi, vannansamlinger på feltet og plantedekke. I tillegg til registrering i feltloggboken, dokumenteres også disse forholdene ved bruk av digitalkamera.

I denne runden skal det sprøytes, og da er det viktig å registrere parametere som mengde sprøytet, (hastighet på traktoren), trykk på sprøyta, lengde på sprøytebommen og evt. om det er mye avdrift under sprøyting.

8. Utstyrsliste

- Jordbor
- Slegge
- Tellefirkant (for å anslå dekningsgraden)
- Plastposer og merkelapper.
- Saks
- Kniv
- Skje (2)
- Linjal
- Petriskåler (24 stk.)
- Parafilmstrimler (24 stk.)
- Engangshansker
- Plastflaske (3 stk., ca. 60 ml)
- Vannfast tusj
- Penn
- Feltkart (se vedlegg)
- Digitalkamera

9. Liste over dokumenter som tas med til feltet:

Feltloggbok med SK34 Registrering av feltdata
Sprøyteplan
KH07 Ekstern prøvetaking og prøvebehandling
SK01 Avviksskjema (2 stk.)

10. Vedlegg

Feltkart for Syverud.

Feltkart Syverud

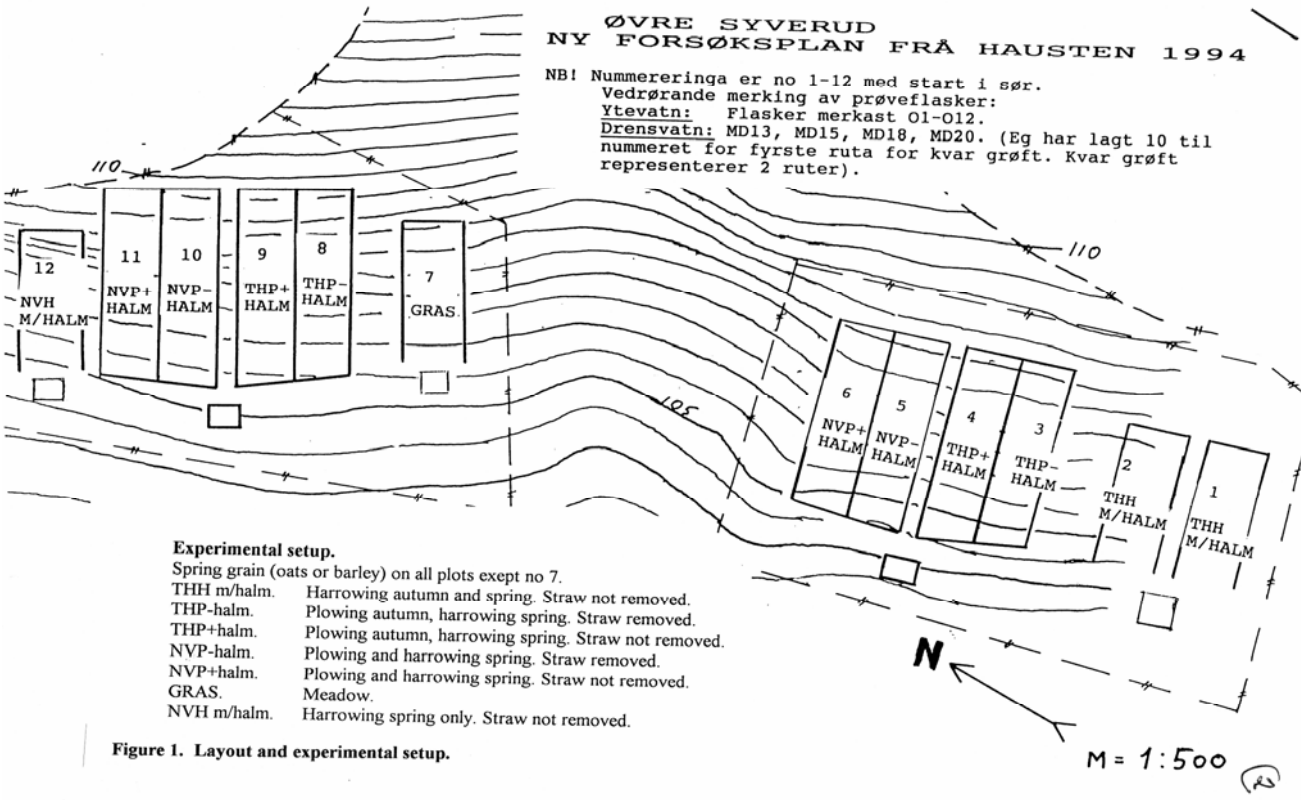


Figure 1. Layout and experimental setup.

Det er rute 3 og 4 som skal sprøytast.
Areal: Rute 3 er 199 m^2, rute 4 er 203 m^2.
Rutene er nummererte slik at den fyrste ruta ein kjem til er nummer 1.